REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1938

1939

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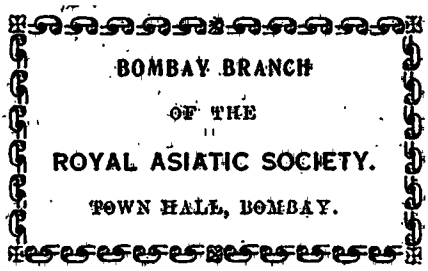

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The Proceedings of the Sixteenth Medical Research Conference held at New Delhi from the 12th to 16th December, 1938, are printed as a separate appendix to this volume and are for the official use of the Association only.

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The Scientific Advisory Board consisted of the following :—

President :

Major-General E. W. C. BRADFIELD, C.I.E., O.B.E., K.H.S., I.M.S., Director-General, Indian Medical Service. (1st January to 26th May and 17th September to 31st December, 1938).

Major-General N. M. WILSON, O.B.E., K.H.S., I.M.S., Offg. Director General, Indian Medical Service. (27th May to 16th September, 1938).

Members :

Colonel A. J. H. RUSSELL, C.B.E., K.H.S., I.M.S., Public Health Commissioner with the Government of India. (1st January to 12th March and 10th June to 31st December, 1938). *Honorary Secretary.*

Lieut.-Colonel E. COTTER, I.M.S., Deputy Public Health Commissioner with the Government of India. (13th March to 9th June, 1938). *Honorary Secretary.*

Colonel J. TAYLOR, C.I.E., D.S.O., V.H.S., I.M.S., Director, Central Research Institute, Kasauli. (1st January to 30th March and 30th July to 31st December, 1938).

Lieut.-Colonel W. J. WEBSTER, I.M.S., Offg. Director, Central Research Institute, Kasauli. (1st April to 29th July, 1938).

Bt.-Colonel R. N. CHOPRA, C.I.E., K.H.P., I.M.S., Director, School of Tropical Medicine, Calcutta.

Lieut.-Colonel S. S. Sokhey, I.M.S., Director, Haffkine Institute, Parel, Bombay.

Lieut.-Colonel H. E. SHORTT, I.M.S., Director, King Institute, Guindy, Madras. (1st January to 26th May, 1938).

Dr. C. G. PANDIT, Offg. Director, King Institute, Guindy, Madras, (27th May to 31st December, 1938).

Lieut.-Colonel G. COVELL, I.M.S., Director, Malaria Institute of India, Kasauli.

Dr. R. ROW, O.B.E., 27, New Marine Lines, Fort, Bombay.

Dr. Jivraj N. MEHTA, Dean, Seth G. S. Medical College, Bombay.

Dr. VISHWA NATH, Assistant Professor of Pathology, King Edward Medical College, Lahore.

Co-opted Members.

Dr. L. E. NAPIER, School of Tropical Medicine, Calcutta.

Dr. W. R. AYKROYD, Director, Nutrition Research, I.R.F.A., Coonoor.

Dr. M. I. NEAL EDWARDS, W.M.S., Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi.

Dr. A. C. UKIL, Officer-in-Charge, Tuberculosis Enquiry, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. FREMODT MOLLER, Superintendent, Union Mission Tuberculosis Sanitorium, Chittoor, Madras Presidency.

Dr. JOHN LOWE, Leprosy Research Worker, School of Tropical Medicine, Calcutta.

Major M. L. AHUJA, I.M.S., Offg. Director, Pasteur and Medical Research Institute, Shillong.

Dr. R. B. LAL, Offg. Director, All-India Institute of Hygiene and Public Health, Calcutta.

Composition of Advisory Committees constituted for the 16th Medical Research Conference and the year 1939.

1. *Cholera.*

Colonel J. TAYLOR, (*Chairman*).
Major W. D. B. READ, (*Secretary*).
Major M. L. AHUJA.
Major C. L. PASRICHA.
Dr. C. G. PANDIT.
Dr. R. B. LAL.
Dr. K. C. K. E. RAJA.

2. *Malaria.*

Lieut.-Colonel G. COVELL, (*Chairman*).
Major M. K. AFRIDI, (*Secretary*).
Major H. W. MULLIGAN.
Dr. K. V. KRISHNAN.
Dr. B. M. DAS GUPTA.

3. *Tuberculosis.*

Major-General E. W. C. BRADFIELD, (*Chairman*).
Dr. A. C. UKIL and Dr. SIKAND, (*Joint Secretaries*).
Dr. FRIMODT MOLLER.
Colonel G. G. JOLLY.
Major-General N. M. WILSON.
Colonel H. STOTT.
Dr. Jivraj N. Mehta.

4 *Maternal Mortality.*

Lieut.-Colonel E. COTTER, (*Chairman*).
Dr. M. I. NEAL EDWARDS, (*Secretary*).
Lieut. R. PASSMORE.
Dr. JHIRAD.
Dr. L. E. NAPIER.
Dr. N. A. PURANDARE.

5. *Nutrition.*

Colonel A. J. H. RUSSELL, (*Chairman*).
Dr. W. R. AYKROYD, (*Secretary*).
Lieut. R. PASSMORE.
Sir BRYCE BURT.
Mr. P. J. KERR.
Dr. G. SANKARAN.
Professor B. N. GHOSH.
Professor S. P. NIYOGI.

6. *Plague.*

Lieut.-Colonel S. S. SOKHEY, (*Chairman*).
Colonel J. TAYLOR (*Secretary*).

Dr. V. R. KHANOLKAR.
Lieut.-Colonel C. M. GANAPATHY.
Dr. R. ROW.
Dr. M. YACOB.

7. *Leprosy.*

Major General H. C. BUCKLEY, (*Chairman*).
Dr. J. LOWE, (*Secretary*).
Lieut.-Colonel G. VERGHESE.
Dr. B. N. VYAS.
Dr. R. G. COCHRANE.
Dr. R. ROW.
Dr. V. R. KHANOLKAR.

8. *Pharmacology.*

Bt.-Colonel R. N. CHOPRA (*Chairman*).
Dr. B. B. DIKSHIT, (*Secretary*).
Captain ILAHI BAKSH.
Dr. B. MUKERJI.

The Board held two meetings during the year on the 12th and 16th/17th December, 1938.

At its meeting on the 12th December, 1938, the Board appointed the various Advisory Committees to which additional members were co-opted for the purposes of the Medical Research Conference. On the 16th/17th December, 1938, the Board considered in detail the programme of research for the year 1939-40 recommended by the 16th Conference of Medical Research. The programme of research recommended by the Board is given later in the report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1938.

The researches, which were carried out during the year under report, and which were recommended by the 15th Conference of Medical Research Workers held at New Delhi from the 29th November to 4th December, 1937, were finally approved by the Governing Body of the Association at their annual meeting held in the Imperial Secretariat, New Delhi, on the 26th February, 1938.

CHOLERA.

1. Cholera bacteriological enquiry, by Major C. L. Pasricha, I. M. S., School of Tropical Medicine, Calcutta.

I. Cholera patients.

During the first half of this year, 1307 samples of faeces from 631 clinical cases of cholera were examined. Agglutinable vibrios were isolated from 58 per cent. of the cases, Hagg vibrios (H agglutinable) and Nagg vibrios (not agglutinable) were isolated from 7 per cent. and vibrios were not isolated from 35 per cent. of the cases. The majority of the vibrios strains belonged to Heiberg's groups I & II. During July, August and September of this year stools from 148 cholera patients were examined. Vibrios were isolated from 16 per cent. of these patients, Agg vibrios (agglutinable) from 10.7 per cent. and Hagg vibrios from 1.3 per cent. As the percentage of vibrios isolated during this inter-epidemic period was low smears of cholera stools were carefully studied. In 3 stool smears vibrios were recognised under the microscope but were not isolated either on bile-salt or on Aronson's medium. There is a real necessity for improvement in the methods employed in the isolation of vibrios.

In fifteen patients in whom repeated stool examinations had yielded negative results the blood was examined for cholera agglutinins. One sample of blood from each patient was collected after the 6th day of the disease. In five of the fifteen patients agglutinins for *V.cholerae* were present. The day of the disease and the dilution at which standard agglutination was visible to the naked eye is given below.

	Inaba antigens	
Day of disease	"H"	"O"
7	320	Nil (in 1 in 10)
9	640	80
9	160	80
9	20	40
10	640	320

In 110 healthy persons, who gave no history of cholera, agglutinins were not demonstrable (in serum dilutions of 1 in 10) with the antigens employed. Thirteen contacts of cholera patients (workers in the cholera wards) had no cholera agglutinins in their sera (1 in 10 dilution).

Bacteriophage active for *V.cholerae* were found in the stools of a number of patients in whom vibrios were not recovered.

Ogawa types. 380 strains of vibrios isolated from 250 patients were studied. Vibrios of the Ogawa type were found in 34 or 13 per cent. From fifteen of these patients both the Ogawa and the Inaba types of vibrios were isolated. One strain was agglutinable by Ogawa O serum and inagglutinable by Inaba H & O serum. This strain is motile.

The reaction of cholera stools. The hydrogen-ion concentration of stools of 150 cholera patients collected during the first two days after the onset of symptoms was determined by the calorimetric method. Approximately 65 per cent of typical rice-water stools without visible faecal matter were alkaline. The majority of these samples had a pH value between 7.5 to 8.5. About 35 per cent of the stools were acid (pH below 7.0 but not below 6.0). Stools containing faecal matter were distinctly acid in reaction.

In 30 cholera patients whose stools were examined daily, vibrios were found in nearly all the alkaline samples and in fewer samples when the reaction of the stool was on the acid side.

Cholera Urines. Vibrios were not isolated from any of the samples of urine collected by catheter from 122 cholera patients. The hydrogen-ion concentration of samples of urine from 50 bacteriologically proven cholera patients was estimated. The urine during the acute stage of the disease was markedly acid. (pH 4.4 to 5.4) and became alkaline as the acute symptoms subsided.

The reaction of the blood of cholera patients during the acute stage of the disease. The pH of the blood of 27 cholera patients was determined by introducing a special electrode into the vein. The pH of the blood was 6.88 to 7.54. This is distinctly below the normal value of 7.4 to 7.8 by this method.

Agglutination for V.cholerae in cholera patients. Sera from 113 cholera patients were examined for the presence of agglutinins (Inaba "O" and "H" & "O" as well as "O" and "H" & "O" antigens prepared from the homologous strains). The results indicate that in the majority of the patients agglutinins are demonstrable from the 7th to 10th day of the disease. The H agglutinins appear earlier and in higher dilutions both for the homologous and the standard Inaba strain.

2. Vibrios from non-cholera individuals.

Vibrios were isolated from 25 or 4.3 per cent. of 575 non-cholera individuals examined. Vibrios were isolated more frequently during the epidemic period (6.6 per cent. of 300 individuals during the epidemic period) and from only 2 per cent. during the inter-epidemic period. Three agglutinable strains of vibrios were isolated from healthy individuals.

3. Vibrios from non-human sources.

The stools of certain domestic and laboratory animals were examined for the presence of vibrios. Vibrios were isolated from the faeces of horse, buffalo, cow, sheep, goat, pig, rat, guinea-pig, rabbit and monkey and were not found in the faeces of mule, cat and mouse.

The vibrios isolated were inagglutinable with Inaba O serum but 44 per cent of the strains were agglutinable with Inaba H & O serum.

4. Vibrios from natural waters.

Vibrios were isolated from 89 or 70 per cent. of the 128 samples of water collected from different sources. According to Heiberg's classification 178 colonies from the 89 samples were grouped as follows :—63 in group I, 104 in group II, 2 in group III, one in group IV, one in group V and 7 in group VI. All strains were inagglutinable with Inaba O serum and only 10 per cent. showed H relationship.

The seasonal variation and the characteristic of vibrios isolated from certain waters in hyper-endemic areas in Calcutta has recently been undertaken. Hagg vibrios were isolated from 5 of the 30 samples of such waters examined. Chemical analysis shows that the pH of the samples varied from 8.1 to 8.7 the chloride content from 0.8 to 29.2, the total solids from 17.7 to 113.8 and the organic matter from 0.22 to 1.70 per 100,000 parts of water. There is apparently no relationship between the nature of the water and the presence of vibrios.

5. Testing for potency etc. of cholera vaccines.

Fourteen cholera vaccines were tested for sterility, freedom from abnormal toxicity, antigenic response in rabbits and man and protective value in guinea pigs. All the vaccines passed the test for sterility and were free from abnormal toxicity. Six cholera vaccines prepared by recognised laboratories gave satisfactory antigenic response both in man and in rabbits and protected guinea pigs against 2 M.L.D. of *V.cholerae*. Four of the eight commercial preparations produced cholera agglutinins and possessed protective properties and four vaccines gave uniformly negative results.

A second series of cholera vaccines are under test. The cultures used in the preparation of some of these vaccines have been obtained from their manufacturers. At least one manufacturer uses a number of strains, the majority of which are Inaba O inagglutinable.

One strain received from a laboratory was Inaba 'O' agglutinable but only partially agglutinable with Inaba H & O serum. This strain on further study proved to be mainly non-motile. It is hoped to isolate a stable non-motile variant. The strain is not contaminated with the known types of choleraphages and is lysable by all the types, including types A & N whose action is restricted to *V.cholerae*.

6. Vi-antigen.

Sera prepared with living broth cultures of *V.cholerae* absorbed with acid-treated-suspensions of *V.cholerae* possess a residual antibody which agglutinates to low titre a number of cholera strains. The nature of this agglutination has not been determined.

2. Cholera carrier and epidemiological enquiries by Dr. R. B. Lal, at the All-India Institute of Hygiene and Public Health, Calcutta.

The main purpose of this enquiry was two-fold, viz., (i) to test the efficiency of a differential medium for the isolation of agglutinable (Inaba 'O' positive) vibrios from natural sources and (ii) to apply this technique for the isolation of vibrios from epidemic and endemic areas. The differential medium to be tested consisted of a modified form of Wilson and Blair's liquid enrichment medium in which broth was replaced by peptone, mannose was substituted for mannite and brilliant green was omitted. The materials used to test the medium were samples of stools collected from cholera and non-cholera patients, contacts of cholera cases and healthy persons not giving history of recent cholera contact, and of samples of water collected from tanks, wells, rivers and drains in Calcutta and certain villages in Bengal. The preliminary investigations in Calcutta were conducted by Major W. D. B. Read, I.M.S.

In collecting samples of stools a teaspoonful of the material was added to 20 c.c. of 2 per cent NaCl solution contained in glass-stoppered bottles. Two to 3 drops of N/1 NaoH were then added. The quantity of salt solution was later increased to 40 c.c. The interval between the collection of stools and culturing was on an average 4½ hours for Calcutta specimens and 8 to 20 hours for specimens collected in the villages. Samples of water were collected in sterile pint whisky bottles by dipping them directly into the source. These collections were made in duplicate or triplicate, one sample being reserved for chemical analysis and the others for culturing. To each of the latter bottles two teaspoonfuls of table salt were added. For purposes of comparison water samples were also collected in 10 oz. screw-capped bottles containing 20 c.c. of 10 per cent. peptone and 10 per cent. NaCl with 8 drops of N/10 NaoH, the total volume being made up to 200 c.c.

On arrival of the specimens in the laboratory 10 c.c. of the stool emulsion was added to an equal amount of freshly prepared W & B's medium in 2 oz. screw-capped phial. For purpose of control 2 c.c. of the emulsion was added to 20 c.c. of one per cent peptone water contained in the same type of phial. In both cases the pH was brought upto 9.2. The bottles were then incubated for 18 to 20 hours and plated on Aronson's and Agar media. Ten or more likely colonies from each plate were picked up and examined. In the case of the water samples collected in peptone water the contents were adjusted to pH 9.2 and incubated directly. The salted samples were passed through Seitz filter in quantities of 300 to 1000 c.c. (any additional amount required was supplied from the unsalted bottle). The Seitz discs were inoculated into the freshly prepared modified W & B's media. The pH was adjusted to 9.2 and the bottles were incubated for 18 to 20 hours. Subsequently treatment was the same as in the case of stool samples. The results obtained in Calcutta are shown in tables 1 and 2.

Table 1.

Isolation of vibrios from sources in Calcutta using W & B's medium.

SOURCES.	SPECIMENS.								
	Isolations.				Serology.			Mannose.	
Nature.	Number	Number of specimens.	Vibrio positive numbers	Percentage	Inaba O+	Inaba H+	Nag	Fermented.	Not fermented.
Stools from cholera cases	37	45*	13	28.9†	13	0	0	13	0
Stools from healthy individuals & non-cholera patients.	186	186	39	21	0	0	39	29	10
Water samples from tanks, rivers, etc.	78	174	136	78	4	..	132	125	11

* All these samples were vibrio-negative on first examination using peptone water.

† No record of Inaba O negative or Nag vibrios was kept.

Table 2.

Isolation of vibrios from sources in Calcutta using 1 per cent peptone water.

SOURCES.	SPECIMENS.									
		Isolations.			Serology.			Mannose.		
Nature.	Number.	Number of specimens.	Vibrio positive nos.	Percentage.	Inaba O+	Inaba H+	Nag.	Fermented.	Not fermented.	Not tested.
Stools from cholera cases.	35	76	25	32.9	8 (10.5%)	0	17	12	7	6
Stools from non-cholera patients.	106	106	8	7.5	0	0	8	2	6	0
Water samples from tanks, rivers etc.	12	14	14	100	0	0	14	10	4	0

The results of field investigation are shown in tables 3 and 4.

Table 3.

Isolation of vibrios from sources in Rampurhat and Basirhat Centres using W & B's medium.

SOURCES.			SPECIMENS.							
			Isolations.			Serology.			Mannose.	
Centres.	Nature.	Number.	Number of specimens.	Vibrio isolation no.	Percentage.	Inaba O+	Inaba H+	Nag.	Fermented.	Not fermented.
Rampurhat.	Stools from healthy individuals.	66	66	4	6	0	0	4	4	0
Basirhat Group A.	Stools from healthy individuals.	121	121	5	4	0	0	5	5	0
Basirhat Group B.	Stools from cholera convalescents.	3	18	10	55	7	2	1	10	0
	Stools from cholera contacts.	21	74	17	23	4	1	12	14	3
	Stools from healthy non-contacts.	2	2	0	0	0	0	0	0	0
Rampurhat.	Water samples from tanks, rivers, wells, etc.	36	41	2	5	1	1	0	2	0
Basirhat Group A.	Do	38	49	20	40.8	2	5	13	16	4
Basirhat Group B.	Do	8	26	22	78.5	1	3	18	14	8

Table 4.

Isolation of vibrios from sources in Rampurhat and Basirhat Centres using peptone water.

CENTRES	SOURCES	SPECIMENS.								
		Isolation				Serology			Mannose	
	Nature	Number	Number of specimens	Vibrio positive No.	Percentage	Inaba O+	Inaba H+	Nag	Fermented	Not fermented
	Stools from cholera convalescents.	3	18	8	44	5	2	1	8	0
Basirhat Group A.	Stools from cholera contacts	21	74	14	19	4	0	10	12	2
	Stools from healthy noncontacts	2	2	0	0	0	0	0	0	0
Basirhat Groups A & B.	Water samples from tanks	7	23	23	100	0	4	19	16	7

Table 5.

Comparative results of isolations from the same samples using W & B's medium and peptone water.

Serological character	STOOLS (245 samples).					WATERS (23 samples).				
	Vibrios isolated with both media	Vibrios isolated with W & B alone	Vibrios isolated with peptone water alone	Positive with W & B but negative with peptone	Vibrios isolated with peptone but negative with W&B	Vibrios isolated with both media	Vibrios isolated with W & B alone	Vibrios isolated with peptone alone	Vibrios isolated with W&B but negative with peptone	Vibrios isolated with peptone but negative with W & B
Inaba O positive	24	24	17	9	5	1	1	0	1	0
Inaba O negative	25	24	21	8	5	22	14	23	0	8
Total ..	49	48	38	17	10	23	15	23	1	8

The low percentage of Inaba 'O' positive isolations from stools of cholera patients in table 2 is striking. This at once throws doubt on the adequacy of the W & B's medium for purposes of isolation of this type of vibrios. It must, however, be remembered that these samples were found vibrio-negative on first examination with peptone water. Clinical records of these patients were examined and the cases were classified according to clinical assessment taking into consideration the nature of stools, the frequency of purging and vomiting, cramps, specific gravity of blood and presence or absence of pain. From this analysis it appeared that in the case of both these media the isolation of Inaba O positive organisms was more frequent in typical cases than in non-typical ones but there were quite a number of failures in clinically typical cases and that some of the clinical atypical cases also yielded positive results. Since the point at issue here is the relative efficiency of the two methods in isolating vibrios it is not proposed to discuss the adequacy or otherwise of the differential medium in isolating vibrios from all cases. The comparative results given in table 5 show that so far as isolation of Inaba O positive vibrios is concerned the differential medium though not wholly efficient is at any rate better than peptone water medium. Moreover, it has the advantage of suppressing the growth of faecal organisms which is of practical value in isolation. For this reason W & B's medium also shows superiority over the peptone water medium in isolating other types of vibrios also. The advantage is however with the peptone water medium when employed for the examination of water supplies.

Epidemicity and Endemicity.

The next question to which attention was directed was the bacteriological aspect of the problem of epidemicity and endemicity. The results of the statistical investigations had indicated that various localities in the south-western part of Bengal could be classified into four types of areas, viz., endemic areas with high epidemicity, endemic areas with low epidemicity, non-endemic areas with high epidemicity and non-endemic areas with low epidemicity. Joynagar, Bishnupur, Rampurhat and Basirhat

areas were selected as types of the above named four classes of areas respectively. Originally the intention was to study each of these types repeatedly in regular rotation but on account of the limited staff and certain other circumstances it has so far only been possible to conduct the study of the last two areas. The method used for collecting and examining the material as also the results of isolation of vibrios from natural sources have been described in the previous section. Besides these studies an attempt has been made to obtain information regarding certin chemical characters of water sources (*vide* table 6) and the immunity status of the population as measured by agglutinin content (*vide* table 7). Before presenting these results a short description of each area is desirable.

Rampurhat area : (Non-endemic with high epidemicity).

The following three villages were selected :—

1. *Pakhuria-Ramkrishtapur*—Dates of investigation—10.7.38 to 14.7.38. Population—484. Last case of cholera—April, 1938.

2. *Bogtui*—Dates of investigation—12.7.38 to 17.7.38. Population—358. Last case of cholera—September, 1936.

3. *Nishchintapur-Dighirpar*—Dates of investigation—21.7.38 to 31.7.38 and 12.11.38 to 16.11.38. Population—548. Last case of cholera—April, 1936.

In none of these or neighbouring villages was there any evidence of the occurrence of cholera cases at the time of investigation. At a later date (25.11.38) another village named Karra (Population, 298) was included for determining the agglutinin content of blood because in this village cholera deaths had not been recorded for 25 years. In selecting persons for blood examination at Patalichandra and Nishchintapur preference was given to those who were getting their supply of drinking water from the tanks from which Inaba O positive vibrios had been isolated.

Basirhat area : (non-endemic with low epidemicity).

The villages in this area may be sub-divided into Group A and Group B according to their recent cholera history.

Group A.

(1). *Rameswarpur*—Dates of investigation—13.8.38 to 22.8.38. Population—263. Last case of cholera—April, 1938.

(2). *Ramnarayanpur*—Dates of investigation—17.8.38 to 24.8.38. Population—234. Last case of cholera—April, 1938.

(3). *Patilachandra*—Dates of investigation—26.8.38 to 5.9.38 and 31.10.38 to 7.11.38. Population—638. Last case of cholera—November, 1931.

Group B.

(1). *Dakshin-Gulaichandi*—Dates of investigation—7.9.38 to 19.9.38 and 18.10.38 to 27.10.38. Population—229. 3 cases of cholera in August 1938, confined to a single family.

(2). *Kodalia*—Dates of investigation—7.9.38 to 19.9.38 and 18.10.38 to 27.10.38. Population—202. Five cases of cholera in September 1938, confined to a single family.

The results of chemical analysis of water-supplies are shown in table No. 6.

Table 6.

Chemical analysis of water supplies.

Centres.	Source.	No. of samples.	pH (range)	Chlorine parts per 100,000 (range)	Total solids parts per 100,000 (range).	Oxidisable matter (Tidy's Process) parts per 100,000 (range).
Calcutta	Tanks	78	6.8-8.2	2.1-48.6	10.8-150.6	0.23-1.94
	Rivers	9	7.8-8.0	0.8-2.5	11.2-17.0	0.11-0.80
Rampurhat	Tanks	32	7.5-8.0	0.6-5.8	9.4-34.0	0.19-1.64
	Wells	8	7.1-8.0	0.7-9.8	28.4-66.6	0.12-0.87
	River	1	7.4	0.6	18.4	0.87
Basirhat	Tanks	75	7.4-8.1	0.9-9.4	8.8-61.0	0.12-0.88
	Well	1	7.4	7.9	75.2	0.58
	River	1	7.9	2.6	17.8	0.42

Table 6 (*a*)

Results of chemical analysis of water samples obtained from tanks from which Inaba O positive vibrios were isolated.

Centres.	Source.	No. of samples.	pH (range)	Chlorine parts per 100,000 (range)	Total solids parts per 100,000 (range).	Oxidisable matter (Tidy's Process) parts per 100,000 (range).
Nischintapur (Rampurhat)	Dighi	6	7.5-7.6	1.3-1.4	9.4-10.6	0.31-0.51
Patilachandra (Basirhat)	Shibaram Mandal's tank.	10	7.8-8.1	1.0-1.4	10.0-15.6	0.16-0.42
Kodalia (Basirhat)	Mobul Mandal's tank.	7	7.8-8.0	3.7-5.8	20.8-26.3	0.28-0.35

Table 6 gives a very rough description of the chemical characters of water supplies in the various areas but it will appear that practically all water supplies examined are alkaline in reaction. The pH, however, varies within narrow limits between 7.5 and 8. The chlorine content as also the total solids are high, the river-waters as a rule show the least amount and the well-waters the largest amount. Some tanks in Calcutta, specially those situated in industrial areas, exhibited extra-ordinarily high figures for cholera and total solids. The oxidisable matter in the water supplies is as a rule large in amount in all the sources examined. The tanks from which Inaba 'O' positive organisms were isolated exhibited more or less the average picture for the respective areas.

The results of serological examination of samples of blood obtained from healthy non-contacts from three villages are shown in table 7.

Table 7.

Agglutination reaction against Inaba O (titre 25 or higher), of samples of blood taken from healthy non-contacts in three villages.

Village centre.	Number of samples examined.	Number showing positive reaction.	Inoculated group.			Uninoculated group.		Persons drinking water from positive tanks.		Uninoculated but not drinking.	
			Last date of inoculation.	No. examined.	No. showing positive reaction.	No. examined.	No. showing positive reaction.	No. examined.	No. showing positive reaction.	No. examined.	No. showing positive reaction.
Patilachandra (Basirhat Centre).	50	34 (68 %)	Nov. 1931.	12	8 (66 %)	38	26 (68.4 %)	32	21 (65.6 %)	13	10 (77 %)
Nischintapur (Rampurhat Centre).	38	27 (71 %)	April 1936.	27	18 (66 %)	11	9 (81.8 %)	20	18 (90 %)	4	3 (75 %)
Kamra (Rampurhat Centre).	40	4 (10 %)	nil.	nil.	nil.	40	4 (10 %)	nil.	nil.	nil.	nil,

The high percentage of those showing specific Inaba 'O' agglutinins in their blood in the first two villages is striking. In contrast to this the comparatively low figures in the third village are equally interesting. It will also be observed that the percentages of positives are not markedly different in the inoculated and the uninoculated groups nor between those who were using the contaminated tanks and those not using them. It would in fact appear that the general population in the first two villages had experienced subclinical infection sufficiently recently to exhibit antibody reaction, although no cases of cholera were said to have occurred in these villages for a number of years. In Kamra also as high as 10 per cent population was found to give positive reaction against cholera vibrio even though there had been no recorded death from cholera for the last quarter of a century. Arguing from analogy it would appear that the occurrence of subclinical cases or carriers in these villages is fairly frequent. In this connection the results of bacteriological investigations shown in tables 3 and 4, particularly those relating to Rampurhat and Basirhat Group A areas are interesting. In both these areas Inaba O organisms were obtained from certain samples of tank water in the absence of declared cases of cholera though no such organisms could be isolated from human faeces. To what extent failure to obtain positive results is due to inadequate means available at present for isolating these organisms cannot be determined but from the information obtained in Calcutta it would appear that a great deal still remains to be done to improve the efficiency of culture medium.

With regard to the persistence of infection in human guts and in water sources data given in table 8 are of interest.

Table 8.

Persistence of vibrios in stools of cases and contacts and in water samples.

Village Centre.	Sources.	Date & result.	Date & result.	Date & result.	Date & result.	Date & result.	Date & result.	Date & result.	Date & result.
Dakshin-Gulai-chandi (Basir-hat)	Contact.	7.9.38 O+	12.9.38 nil.	21.10.38 nil					
Do	Do	7.9.38 O+	14.9.38 Nag.	18.10.38 nil					
Do	Do	7.9.38 O+	12.9.38 nil	16.9.38 nil	19.9.38 nil	20.10.38 nil	25.10.38 nil		
Do	Case.	7.9.38 O+	12.9.38 Nag.	21.10.38 nil	26.10.38 nil				
Kodalia (Basirhat)	Do	7.9.38 O+	12.9.38 O+	16.9.38 O+	19.9.38 O+	18.10.38 H+ Nag.	20.10.38 nil	21.10.38 nil	27.10.38 nil
Do	Do	9.9.38 O+	12.9.38 nil	16.9.38 O+	19.9.38 H+	21.10.38 nil	27.10.38 nil		
Do	Contact	9.9.38 O+	14.9.38 nil	21.10.38 nil	21.10.38 nil				
Nischintapur (Rampurhat)	Water	19.7.38 O+	24.7.38 nil	26.7.38 nil	28.7.38 H+O±	13.11.38 nil	16.11.38 nil		
atilachandra (Basirhat)	Do	26.8.38 O+	31.8.38 O+ Nag.	2.9.38 Nag.	5.9.38 H+ Nag.	7.9.38 Nag.	14.9.38 Nag.	21.10.38 H+ Nag.	31.10.38 H±
Kodalia (Basirhat)	Do	9.9.38 Nag.	12.9.38 O+	14.9.38 Nag.	16.9.38 H+	19.9.38 H+	18.10.38 Nag.	21.10.38 Nag.	

The above findings suggest that :—

1. Infection with Inaba O vibrios by itself may not necessarily cause disease. This may be due either to loss of virulence by the organisms without loss of agglutinability or it may be due to a high degree of immunity attained by the population on account of frequent subclinical infection.

2. To explain endemicity it is necessary to investigate the factors connected with persistence of infection of Inaba O vibrios in man and in water-sources.

With regard to the first point occasional occurrence of severe outbreaks in highly endemic areas would suggest that naturally acquired immunity does not furnish complete explanation and some role must be assigned to either change in virulence or in quantum of infection. With regard to the second point the chemical character of the water supplies may be of importance but the present studies are not sufficient to provide the necessary information.

3. Cholera statistical enquiry by Dr. R. B. Lal, at the All India Institute of Hygiene and Public Health, Calcutta.

In last year's report it was mentioned that for the purpose of studying the epidemiological characteristics of different areas in respect of cholera the *thanas* in the south-western portion of Bengal were combined into nineteen homogeneous cholera districts. During this year detailed studies of these districts were taken up. A summary of these studies is given below.

INFLUENCE OF METEOROLOGICAL FACTORS ON CHOLERA INCIDENCE.

It is well known that cholera, like many other diseases, follows a seasonal cycle. The factors responsible for the seasonal variation of cholera, however, are not well understood. The investigations of Rogers and of Russell and Sundararajan suggest a close association between the seasonal behaviours of cholera incidence and certain meteorological factors. This subject has been examined in the case of five homogeneous districts for which meteorological records are available. Four meteorological factors have been taken into consideration, viz., temperature, rainfall, relative humidity and number of rainy days. Co-variance analysis has been carried out for each of these factors and cholera mortality rate over a number of years. Thus co-variance from year to year and from month to month were separated from the total co-variance and the residual co-variance was obtained. A statistically significant residual co-variance would suggest a real influence of the meteorological factor concerned on cholera incidence. The results, however, indicate that in four out of the five homogeneous districts the incidence of cholera is independent of the meteorological factors. In the case of the fifth district there is a positive correlation with temperature only. (See Table I below).

Table I.

Co-variance analysis of cholera mortality and meteorological factors in homogeneous districts.

Homogeneous District No.	Source variation.	Degrees of freedom	Correlation of cholera mortality with			
			Dry bulb temperature at 8 hours.	Rainfall	Relative humidity at 8 hours.	Rainy days.
IV	Month	11	-0.2949	-0.5212	-0.7584*	-0.5479
	Year	30	0.1014	0.0044	-0.1319	-0.9128
	Residual	341	0.1123*	0.0419	-0.0127	0.0517
VI	Month	11	-0.8088*	-0.8258*	-0.5309	-0.8256*
	Year	30	0.2749	0.1719	-0.0636	-0.0177
	Residual	330	0.0843	-0.0289	-0.0802	-0.0208
VII	Month	11	-0.7681*	-0.8105	-0.6089*	-0,8136*
	Year	30	-0.4070*	0.1185	-0.5298	-0.1033
	Residual	330	0.0942	0.0034	-0.0318	-0.0377
VIII	Month	11	-0 7490*	-0.8022*	-0.0142	-0 6949*
	Year	30	0.0651	-0.1571	0.2528	-0.0012
	Residual	330	0.0639	0.0457	0.0716	0.0141

* Correlation statistically significant.

The validity of Roger's hypothesis of a critical level of absolute humidity, above which conditions become favourable for cholera, has also been investigated. The frequencies of occasions on which (1) cholera mortality was rising or (2) cholera mortality was constant or falling in a given place were distributed in relation to values of absolute humidity rising above or falling below 0.40 mm., which is Roger's critical level. Statistical tests were applied to find out if there was any significant association between corresponding happenings. This study was carried out for Calcutta, Gorakhpur, Saharanpur, Sialkot and Lahore. The results which are set out in the following table throw doubt on Roger's hypothesis.

Table 2.

Association of cholera mortality with Roger's critical level of humidity.

Area.	Province	Probability of chance of occurrence	Remarks
Calcutta	Bengal	0.44	
Gorakhpur	United Provinces	0.01	A positive correlation is suggested.
Saharanpur	-do-	0.03	
Sialkot	Punjab	0.54	
Lahore	-do-	0.22	

DECLINE OF CHOLERA MORTALITY.

A straight line was fitted by the method of least squares to cholera mortality rates of each district for the period 1901-1932. All the nineteen districts showed decline, but it was significant only in ten cases.

HOMOGENEOUS CHOLERA DISTRICTS IN RELATION TO THE RIVER SYSTEM.

It was found that the homogeneous cholera districts do not bear any direct relationship to the river system.

ENDEMICITY AND EPIDEMICITY.

The system of classification of the homogeneous cholera districts in regard to their endemicity has been further elaborated. The criterion for judging the endemicity of an area previously taken was the relative periods of absence of cholera mortality in successive months. Since the size of population and number of component units vary greatly with different homogeneous districts and these factors have an obvious bearing on the above classification it was considered desirable to remove their influence. This was done by working out multiple regression equations for the above three variables, and then taking the deviation of the observed frequencies of different sized periods from the expected values for purposes of comparison. The classification obtained by this method showed that the endemic regions are mostly situated along the lower reaches of the Hooghly. (See table 3 below).

Table 3.

Classification of homogeneous districts into endemic and non-endemic areas with varying degrees of epidemicity.

Homogeneous District No.	Endemicity.	Degree of Epidemicity.
1	Non-endemic	Moderate
2	do.	Moderate
3	do.	Moderate
4	do.	Moderate
5	do.	Very low
6	Endemic	Low
7	do.	Moderate
8	do.	High
9	Non-endemic	Very low
10	do.	Very low
11	do.	Low
12	do.	Very low
13	Endemic	Low
14	do.	Moderate
15	do.	Very low
16	do.	Low
17	do.	High
18	do.	Very low
19	Non-endemic	Low

The analysis has been repeated by taking area as a variable instead of the units composing the districts but the correlations obtained are less significant in a majority of the instances.

A further classification of the homogeneous districts according to the varying degrees of epidemicity was obtained on the basis of the contribution of the yearly variance to the total variance in each case.

Description of homogeneous cholera districts.

An endeavour was made to study the importance of such factors as physiographical features, climate, movement and density of population etc., as could be found in District Gazeteers in determining the epidemiological characteristics of the cholera districts. Relevant information was only available for eleven districts, and the remaining eight districts consisted of single *thanas* only. This study has failed to bring out any definite relationship between the different characteristics of an area.

Forecast.

As a step towards devising a suitable method of forecasting, periodicity of cholera incidence in each homogeneous district was investigated by means of Fosters' Grating Periodograph. For this purpose thirty-two years' figures were taken. In none of the nineteen homogeneous districts could any periodicity other than one due to season be demonstrated.

The new method of forecasting cholera mentioned in last year's report, which has given fairly good predictions for Calcutta for three successive years, viz., 1936, 1937 and 1938, was applied to the homogeneous districts. The method proved successful for ten homogeneous districts but not for others.

4. Enquiry into the cholera vibrios and related vibrios under the Director, Central Research Institute, Kasauli.

A. Serological diagnosis of *V.cholerae.*

The investigations on the incidence of vibrios in water and their characters, especially in non-endemic areas, the results of which had been reported at the last conference were published in full in the April 1938 number of the Indian Journal of Medical Research. The information obtained had furnished further evidence as to the necessity for separate determination of O agglutination for the diagnosis of *V.cholerae* and the findings were embodied in a communication to the May Session 1938 of the Office International d'Hygiene Publique along with other evidence on the point. It was shown that in a series of 160 strains isolated in non-endemic areas of the Punjab 38% were H agglutinators and that these included strains of O serological types similar to many of those isolated from cases of clinical cholera and carriers in Bengal. The almost universal presence of vibrios in natural open water sources in India which has been demonstrated was considered to indicate the probable source of origin of vibrios of types (other than O group No. I) which have been isolated from healthy individuals and even from cholera cases, and H agglutinators may be included amongst such strains.

The Cholera Commission at the Session of May 1937 in Paris had not fully accepted that O agglutination should be the basis of diagnosis of *V. cholerae.* On further consideration and on the basis of the communication submitted to the May Session of 1938 the Cholera Commission recorded the following opinion :—

"Since the H element of the *V.cholerae* is common to many types of water vibrios the isolation from stools of cholera cases of vibrios which agglutinate with a serum containing H agglutinins does not in consequence justify diagnosing them as *V.cholerae.* The test of O agglutination, on the other hand, defines the vibrios to which a definite cholerigenic role can be assigned."

B. Differential isolation of the vibrios.

It has been found that under certain circumstances the isolation of *V.cholerae* in the presence of other vibrios is difficult. Many factors appear to be concerned in this and it appears that, when enrichment methods are employed, more vigorously growing vibrios may outgrow the cholera vibrio. The finding of the very common presence of vibrios in water indicates that, if accurate information as to the incidence of *V.cholerae* in water is to be obtained, improved methods of technique must be employed. Such improved methods are necessary for any field enquiry which may have as its object the determination of the incidence and persistence of the cholera vibrio in waters in endemic areas.

An enrichment medium for the purpose must fulfil the following objects :—

(a) suppression of growth of other organisms such as *Proteus* and those of the *coli-aerogenes* group.

(b) producing differential growth of *V.cholerae* as compared with other vibrios.

Investigations on the subject have constituted the larger part of the work of the Cholera enquiry at the Central Research Institute during

the year. Many different methods have been tried. The first line which gave some indication of being of value was the use of a peptone water medium containing 1% of mannose. When an inoculum of *V.cholerae* along with one of a non-mannose fermenting vibrio was used in this medium the mannose fermenting *V.cholerae* rapidly outgrew the opposing organism and it could be recovered even when the original inoculum of *V. cholerae* in relation to the other strains was as low as 1 to 40,000. The principle of separating vibrios on the basis of their sugar fermentations appears to be a general one and arabinose fermenters can be separated in the same way from non-arabinose fermenters by the use of an arabinose peptone water.

When this principle was applied to cultures in which organisms of the coli-aerogenes group were also present or to faecal suspensions the fermentation of mannose by coliform organisms also resulted in rapid production of acid, and isolation of the vibrio was difficult or impossible when small inocula were used, even when the initial pH was 9.4. If the enrichment was carried out in screw-capped bottles and the pH corrected at intervals throughout the day, by the addition of alkali, recovery was in many cases possible.

The use of such a method was not found practical and other lines were tried.

Considerable success was obtained by means of a modification of the liquid enrichment medium of Wilson and Blair in which peptone water was substituted for broth, and mannose for mannite, and a mixture of salts at 2% concentration in character and proportions similar to those existing in sea water. The details of the preparation of this medium are :—

Prepare 1% Witte's peptone and autoclave ..	100 c.c.
Add with sterilised pipettes	
Sea salt mixture	6 c.c.
Mannose 10%	5 c.c.
Liq. bismuthi (W & B)	6 c.c.
Sodium sulphate (Excise) 20%	6 c.c.
Absolute alcohol	1 c.c.
Hg Cl 1/10,000	4 c.c.

Adjust pH to 9.2 after adding inoculum. The total volume of inoculum and peptone should be arranged to amount to 100 c.c. This was conveniently done in the case of stools by adding 50 c.c. of stool emulsion to 50 c.c. of 2% peptone. None of the ingredients need sterilization except the peptone.

This was used immediately in screw-capped bottles. This medium was found to suppress growth of *coli-aerogenes* group organisms and *Proteus* while permitting growth of vibrios when these and other organisms in faeces were present. In screw-capped bottles to which *V.cholerae* had been added along with those other organisms or a faecal suspension only a slight reduction of alkalinity occurred and the vibrio could be isolated.

With this medium an estimated count of 3 vibrios could be isolated in the presence of 5.0 c.c. of soft stool, while 30 vibrios could be isolated in the presence of the same stool inoculum and 95,000 non-mannose fermenting inagglutinable vibrios. Similarly 3 vibrios could be isolated

from 3 litres of water in the presence of 11,000 non-mannose fermenting inagglutinable vibrios and 107,000 coliform organisms.

The medium however will not differentiate agglutinable vibrios and mannose fermenting inagglutinable vibrios.

A field trial of the methods which had been investigated in the laboratory was undertaken in Calcutta in April and May by Major Read, I.M.S.

Seventy-six specimens from 37 cases of clinical cholera which had proved negative on first examination by the ordinary methods in use were examined by means of both the bismuths sulphate medium and ordinary peptone water enrichment, 28.9% proved positive by the new method and 15.3% by peptone water enrichment. Nine of the 37 were shown to be positive by the bismuth sulphate method which would have been declared negative by the peptone water method. On the other hand 5 cases were negative by the new method and positive by peptone water. Of the latter 4 occurred on one day and there is a possibility of a technical error. Fifteen out of 35 cases gave inagglutinable vibrios and these were present along with the agglutinable vibrio in 6 cases.

Inagglutinable vibrios were isolated from 7.5% of the patients in the Carmichael Hospital and 25% from those in the Campbell Hospital. Little difference was shown by the use of the two methods but the bismuth sulphate method selected mannose fermenting vibrios while 6 out of 8 strains obtained by peptone water enrichment were non-mannose fermenters.

By the new method agglutinable strains were isolated from 4 out of 84 water sources in the suburbs of Calcutta but no comparison was made with other methods. A high degree of suppression of all coliform types was obtained which was an important aspect of the results. The use of the same method has been continued in Bengal and reports of a series of successful isolations of the O agglutinable vibrio from tanks in rural areas have been received. Communications from other workers have shown some differences in findings and in some cases the bismuth sulphate medium has appeared to cause almost complete inhibition of growth of vibrios as compared with peptone water.

The various factors in the medium have accordingly been studied including the concentration of bismuth, sodium sulphite, alcohol and perchloride of mercury. The results appear to vary according to the amounts of faecal matter present and the organisms of different types present. It has been found that a concentration of Liquor Bismuthi to the extent of three times that employed in the original formula improves the isolation from faeces and reduces to some extent the inhibitory action of the medium on *V.cholerae* in high dilutions of cultures.

Later studies on the action of salts on the value of the medium have shown that when a salt mixture of composition equivalent to that of sea salt is used in a proportion of 2 per cent. in place of 0.5% NaCl originally used, isolation is greatly improved and the inhibitory action of the medium is abolished. A comparison with 2% NaCl shows some increased value of the salt mixture. From 5 c.c. of stool which contained coliform organisms in such an amount that 0.2 c.c. of a 10^{-6} dilution grew in MacConkeys' broth with production of acid and gas added to 25 c.c. of the medium along with 0.4 c.c. of a 10^{-8} dilution of overnight broth culture of *V.cholerae* (estimated count =0.14 vibrios per c.c. of medium) the *V.cholerae* was recovered with ease from an ordinary agar plate. This inoculum of the

V.cholerae strain was the limit at which growth in broth could be obtained in a control. This improved method has not yet been submitted to a field trial but appears likely to be of high value.

The difficulty due to presence of mannose fermenters of types other than *V. cholerae* in water still remains.

C. STUDIES ON FERMENTABLE CARBOHYDRATES.

Dr. S. P. Bose was appointed to carry out chemical studies for the enquiry and has undertaken the preparation of various substances with a view to possibly obtaining one which is specifically fermentable by the agglutinable vibrio.

The first studies were made on extracted polysaccharide fractions of the smooth and rough forms but no encouraging results were obtained on this line. A series of preparations was next made with mannose as a base. Mannonic acid was extracted and the Na, K, Ca, Mg, Cd, and Li salts of this prepared. Methyl derivatives, osazones, hydrazones and diacetones and amino compounds were also prepared. The fermentation of these preparations by *V.cholerae* and other mannose fermenters and non-mannose fermenters was tested. Certain of these compounds which retain the aldehyde group were not fermented and others in which this group was absent were fermented. The majority were soluble in water but some caused a precipitation when sterilized in peptone water solution. None of the preparations so far made have been differentially fermentable by the agglutinable vibrio as compared with other mannose fermenters. Further preparations based on the potassium salt of mannonic acid are being made and work is being continued on the same lines.

D. The objects for which the purely laboratory investigations were originally taken up at the Central Research Institute have now been largely accomplished. It is considered that the basis which has been obtained should now be applied to the field investigation of the endemiology and epidemiology of cholera and the enquiry at the Institute on its present basis will terminate in March 1939. The institute will continue to cooperate in the work on certain lines and, in accordance with such arrangements as may be made for field enquiries, may ask for a small grant for expenses but not for staff.

5. Cholera enquiry under Dr. S. R. Pandit at the Pasteur Institute, Shillong.

The Goalpara district being almost the gateway to the Assam Valley, this district was selected for field enquiry. The main object of the enquiry was to estimate in a rural population, the incidence of vibrios in man and his environment, before, during and after an epidemic of cholera.

The unit, consisting of a Field Medical Officer, a Field Bacteriologist, a laboratory attendant and a sample collector arrived at Dhubri, the headquarters of the district, in the last week of June.

At this time there was little or no cholera in the district but the rivers were in high floods and with the receding of flood waters, cholera as usual was anticipated. In order to ascertain the incidence of vibrios in normal times before an epidemic started, a rural area adjoining Dhubri was selected for examination of stools of healthy individuals and of natural water sources used by them. This area consists of 4 villages and has a population of just over 4,000. Water for drinking and domestic purposes is drawn chiefly from shallow wells. This area has been free of cholera.

The first cases of cholera were reported on July 28th—10 attacks and 8 deaths, from 2 villages in the Bilasipara thana on the bank of the Brahmaputra. The epidemic spread to both banks and both subdivisions of the district and is still continuing. Up to the middle of November, for which figures are available, there have been about 2,700 attacks, and 1,600 deaths. Specimens of stools from cases, convalescents, contacts and healthy individuals and of water have been examined from 12 different cholera-affected areas.

The methods of cultivation employed were the usual alkaline peptone water enrichment. The modified Wilson-Blair medium has not been tried. During the period up to the middle of November 1,202 samples were examined at the Unit laboratory, of which 346 were from the epidemic areas and 856 from the cholera-free area.

Epidemic areas.

Out of 346 examinations, 46 were from cases, 27 from convalescents, 73 from contacts, 20 from water sources and 180 from healthy individuals.

Cases. From specimens of 46 cases, the agglutinable vibrio was isolated in 28 and the inagglutinable vibrio from 7. Four of these were of type VI (all Nag) and 3 of type I (1 Hag and 2 Nag). Nag and Hag refer to inagglutinable vibrios which do not and do agglutinate with the flagellar antibody of Inaba.

Convalescents. 27 stools of convalescents were examined, vibrios being isolated from 7. One was an Ag vibrio, one of type III (Nag), one of type V (Hag) and 4 of type VI (all Nag).

Contacts. Stools of 73 healthy contacts were examined. Type VI vibrios were isolated in 2 (both Nag) and the agglutinable vibrio in 3 others. None of these contacts developed the disease. A repetition of culture in 2 of these positive contacts on two further occasions gave negative results. Two of the Ag. strains were non-haemolytic but the third was partially haemolytic.

Water. Twenty samples were examined, of which 8 (40%) were positive. Three strains were of type I (2 Hag and 1 Nag), 2 of type II (both Hag) and 3 of type VI (all Nag). The agglutinable vibrio was not isolated from any source.

Healthy individuals. Stools of 180 individuals were examined. Only 3 (2%) were positive. Two strains were of type VI (Nag) and one of type I (Hag).

The Cholera-free area.

One hundred and seventy-seven samples of water and stools of 679 healthy individuals were examined.

Water. Of the 177 samples, 53 (30%) were positive for vibrios; 32 vibrios were of type II (30 Hag and 2 Nag), 2 of type I (1 Hag and 1 Nag) and 19 of type VI (all Nag).

Healthy individuals. Stools of 679 healthy individuals were examined. Eight (1.2%) were positive, all the strains being of the non-fermenting type VI (Nag).

Non-cholera stools. During the past year 322 specimens of non-cholera stools have been examined for presence of vibrios by the alkaline peptone water enrichment. These specimens were received at the Pasteur

Institute for routine examinations for dysentery, enteric and helminthic infections etc.

Fifty-eight (18%) were positive, of which 40 were of the non-fermenting type VI, 3 of type I, 6 of type II, 3 of type III, 1 of type VII and 5 of the fermenting type VI.

The frequency of isolation of the inagglutinable vibrio from stools of healthy individuals, cases, convalescents, from non-cholera stools and from water.

From stools of 859 healthy individuals the inagglutinable vibrio was isolated 11 times (1.3%), from 46 cases 7 times (15.2%), from 27 convalescents 6 times (22.2%), from 322 non-cholera stools 58 times (18%) and from 197 samples of water 61 times (31%).

From these findings the conclusion may be drawn that inagglutinable vibrios are natural inhabitants of water and that such vibrios taken in with water, have little chance of survival in the gut of healthy individuals but that in those with an intestinal disturbance, they are more easily to be isolated from stools. Even if the non-fermenting type of vibrios be excluded from these figures, this does not affect the conclusions arrived at.

The non-fermenting vibrio.

Over 50 non-fermenting strains have been examined for various reactions. Unlike vibrios of other fermentation types, none of the 12 cholera phage types acts on them and none is agglutinated by the 'H' antibody of the cholera vibrio; unlike other types, the vibrio shape in this type of organisms is almost invariably absent, the organisms being either monotrichate or lophotrichate, with a bunch of 4-6 flagella. This group of organisms appears to have a greater affinity to the Pseudomonas group, whose fermentative property is low, than to the Vibrio group.

Cholera outbreak at Manipur.

At the request of the Secretary to His Excellency the Governor of Assam, a bacteriological investigation of an outbreak of cholera in Manipur was undertaken towards the end of April 1938. After an absence of cholera for several months, 2 attacks and 2 deaths from cholera were reported during the week ending 2nd April, 1938. When the investigation started there had been 105 cases with 61 deaths during a period of about 3 weeks. Cases were occurring over a wide area in the Manipur Valley but there was no crop of cases of any size from any single village or group of houses. During the 10 days period of investigation stools of only 19 cases were available for cultural examination. The method of cultivation was the usual peptone water enrichment followed by plating on bile-agar plates. The agglutinable vibrio could not be isolated from any of the stools. An inagglutinable vibrio of fermentation type I was isolated from 2 of the cases and a non-fermenter from 3 others.

Blood sera of 12 convalescents were put up for agglutination with suspensions of Inaba, Ogawa and Inaba Rough organisms. The samples were taken on an average about the sixteenth day after the onset of symptoms and not earlier than the eighth. Four of them gave a history of previous cholera inoculation, of whom two had the inoculation a few days prior to the attack. These were positive 1 in 500 with Inaba suspension and about 1 in 250 with Ogawa. A third that had been inoculated a year previously gave a titre of 25 and 50 for Inaba and Ogawa respectively and the fourth, inoculated 3 years previously, was negative 1 in

25. The remaining 8 convalescents had never been inoculated. Of these, 7 were negative 1 in 25 with all the three suspensions but the serum of the eighth agglutinated Inaba smooth suspension up to a dilution of 2,500 and was negative 1 in 25 with the other 2 suspensions. The titre of 2,500 appears to be very high even for a proved case of cholera. The serum had undergone considerable haemolysis and it is not known whether this may have been responsible for any non-specific agglutination. An interesting point is that on absorption with Inaba, there was no diminution of the titre to this organism. A second specimen of the convalescent was not obtainable. Thus with this exception, the sera of all the convalescents that had not been inoculated were uniformly negative.

Cases continued to occur in Manipur for a period of 20 weeks resulting in a total of 755 attacks and 376 deaths.

The negative cultural and serological findings from a series of cases, not too small, and at the commencement of an outbreak of clinical cholera, raises the question whether the vibrio can at times be infective in a less easily cultivable form, say the filtrable or G form of Hadley and others.

THE EFFECT OF BACTERIOPHAGE ON THE AGGLUTINABLE VIBRIO.

To determine what modifications are brought about in the cholera vibrio by the collective action of a large number of phage types, the stock 12-1 phage resistant cultures and the strain resistant to all the 12 phages kept up at the Pasteur Institute, were examined. They were derived from 653, an Ogawa strain, and have been kept phage resistant to fewer or more phage types as they were discovered, for over 5 years. The results of examination are given below :

Motility. Three cultures 12-A, 12-G, and 11-H had nearly the normal motility of the cholera vibrio, motility was diminished in 3 others and was absent in the rest.

Morphology. With the exception of 12-A, all the cultures had atypical morphology, e.g., cocoid forms, large globoid forms and filaments. The filaments, some of which had a spiral shape and were of variable length, were often found in clumps.

Fermentation reactions. Using the usual 6 sugars (in peptone water) and noting the reactions for 3 days, it was found that glucose was fermented by all the 13 cultures, lactose was fermented late by some, but arabinose was not fermented by any. With regard to the other 3 sugars, mannite, saccharose and mannose, there were differences and the same result was not always obtained on repetition. It appears as if phage is responsible for the variable results, for in the case of mannose where there was discrepancy, when the cultures were grown on 3% agar containing this sugar and an indicator, fermentation was obtained, though absent in the fluid sugar medium.

Serological reactions. Suspensions of the cultures in formalised 0.3% saline were tested with Inaba H and O, Ogawa O and Inaba Rough O sera. All the cultures were agglutinated by Inaba Rough O serum in a dilution of 25-100%, with the exception of 3 ; 12-A and 12-M were negative 1 in 50 and 12-B agglutinated to 5%. With Ogawa O serum all the cultures were either negative or agglutinated to less than 5%, the exception being 12-A, which agglutinated to 40%. With H and O serum, with the exception of 3, all were negative, 12-G, 12-A and 12-F giving 100, 10 and 1% agglutination respectively. 12-B and 12-M were practically inagglutinable with all the 3 sera.

It appears from the results that all the cultures with the exception of 12-A, had undergone a definite change to rough and 12-B and 12-M had perhaps changed still further.

In order to see what effect phage has on an agglutinable vibrio when added to water, a flask containing about two litres of water, a little garden earth and a small quantity of stool was autoclaved and was inoculated with 10 c.c. of a 24-hour peptone water culture of a recently isolated cholera vibrio and 5 c.c. of therapeutic phage. In two such experiments, it was found that an agglutinable vibrio of fermentation type I could be recovered for 7 and 17 days respectively, after which no growth resulted in cultures. The duration of life of the vibrio in the water was cut short by the phage, but it had not undergone any recognisable change.

SELECTIVE CULTIVATION OF THE AGGLUTINABLE VIBRIO FROM MIXTURES OF THIS AND THE INAGGLUTINABLE VIBRIO.

Taking a mixture of Inaba and an inagglutinable water vibrio of fermentation type II, the following methods of cultivation were not found superior to the not very efficient peptone water method for the selective isolation of the Ag. vibrio.

(1) Peptone water with a pH higher than 7.6.
(2) Growth in peptone water in an atmosphere of 10-20% CO_2.
(3) Peptone water to which is added sodium sulphate in concentrations of 0.025 and 0.1% or washed R.B.Cs.
(4) Growth in peptone water under anaerobic conditions.
(5) Growth in a heat-killed peptone water culture or filtrate of a peptone water culture of the inagglutinable vibrio.

It was however found that by cultivating the mixture in peptone water and plating on successive days there was a better chance of ioslating the Ag. vibrio on the second or third day; thereafter, there was a tendency for both the Ag. and Nag to roughen and for the Nag to again preponderate.

The selective action of Wilson-Blair medium for the enteric group of organisms is said to be due, in great part, to brilliant green that forms part of the medium, and it seemed likely that a similar substance could be found, having a selective action on the Ag. vibrio as against other Nags. Over 30 substances were tried, chiefly dyes. The list also included some inorganic substances like mercury perchloride and proprietary substances like proseptin, a sulphonamide preparation, spirocid etc. Serial dilutions of each substance in peptone water, pH 9.0, were made in duplicate and a standard loopful of an Ag. vibrio was added to the tubes of one row and of an Nag water vibrio to tubes of the other row, the two suspensions being standardised to the same opacity. The presence or absence of growth was noted after 24 and 48 hours incubation. None of the substances was found to be more inhibitory to the Nag than to the Ag. vibrio.

VIABILITY OF THE CHOLERA VIBRIO IN NATURAL WATER SOURCES.

Two experiments were performed, where a culture of a recent agglutinable vibrio was added to a natural collection of water after its salinity had been adjusted, cultures being made daily thereafter for recovering the Ag. vibrio. Peptone water enrichment and the modified Wilson-Blair fluid medium were the cultural methods employed. In the latter case about 500 c.c. of the sample was filtered through a Seitz disc and this was mashed into 40 c.c. of the medium.

In the first experiment, the water source was a shallow well 3 ft. in diameter with about 3 ft. of water. The pH was 5.1 and salinity 0.5 parts Cl per 100,000. After adding common salt the salinity had increased to 26 parts, the pH being the same. On adding 100 c.c. of the cholera culture, the vibrio could not be recovered in 24 hours or thereafter. It will be noted that pH was very low. A rapid diffusion of salt was also noticed. There was no natural vibrio in the water.

In the second experiment, the agglutinable vibrio was added to a tank 32 ft.×30 ft. The pH of the water varied between 6.3 and 6.7 on different days, the organic matter (Tidy) being 0.12 parts per 100,000. The salinity was adjusted to 70 parts per 100,000 and a litre of the culture was added to an estimated quantity of 56,000 litres of water in the tank. The agglutinable vibrio was recovered in the 24 hours sample but not thereafter. No growth of any kind resulted in the Wilson-Blair medium throughout and in both experiments.

As this work was done during the last week of October and the middle of November, when low temperatures prevailed, it is not possible to say to what extent this factor contributed to the shortness of the period of viability of the vibrio.

6. Cholera enquiry under the Director, King Institute, Guindy.

1. Distribution of the types of V. cholerae.

A more precise determination of the conditions, under which the types of *V. cholerae* occurred in the presidency, was one of the main objects of the Enquiry during the year under report. It was mentioned in the report for 1937 that the presidency could be divided broadly into two zones corresponding to the greater prevalence of the Inaba or Ogawa type. The year started with a general decline of the incidence of cholera nearly all through, and of 107 strains of *V.cholerae* examined in the first quarter of 1938, 99 (including 53 from the city of Madras) were Inaba. An interesting observation at this stage was the occurrence of Inaba strains in South Arcot, Tanjore and Ramnad Districts which had been classed previously as the Ogawa zone. This corresponded with a period of low incidence of cholera in these areas : there was no general outbreak ; the cases were mostly sporadic or limited to a few villages. In the second quarter, there was an increase of cholera in the Northern Circars and the Ceded Districts, the first outbreak following the Sri Rama Navami festival in Bhadrachalam. Only sporadic cases were reported from the Tamil districts. 62 strains examined were all of the Inaba type. Of 98 strains examined in the third quarter, 93 were derived from the Northern Circars and Ceded Districts and were all Inaba. Four strains received from Tanjore during the last week of September were all Ogawa. This change back to Ogawa in the Tanjore District is interesting to note, especially as it occurs at a time shortly preceding the usual winter outbreak, which one expects towards the end of the year. There have been only 16 cultures (of which 12 were from Anantapur and Bellary) examined so far in the last quarter. All were Inaba.

As a rough generalisation it may be stated that while all the strains of *V.cholerae* isolated from the Northern Circars and Ceded Districts, where there were outbreaks of usual severity, were all of the Inaba type consistently with the observations of previous years, the few strains of vibrios

derived from sporadic cases or small village outbreaks in the southern districts were, unlike in previous years, also of the Inaba type. This change in the southern or Ogawa zone occurred during a period of low incidence. It would be of interest to carry out more intensive observations in the Cauvery delta with a view to determining the conditions under which this change occurs and whether the relation between low incidence and the change in the type of vibrio is only accidental or really significant.

2. ISOLATION OF VIBRIOS FROM NATURAL AND OTHER SOURCES OF WATER.

This was continued during the year, using the same technique as reported before. 145 samples of water from different sources were examined and vibrios isolated from 100. Of the 45 negative samples 39 were either chlorinated or treated otherwise. 300 strains of vibrios from these 100 sources were further examined. None were agglutinated by a group I 'O' serum. Their distribution in the Heiberg scheme was as follows :—I-113, II-139, III-2, IV-2, V-17, VI-10, VIa-17.

3. SEROLOGICAL GROUPING OF NAG VIBRIOS.

With a view to making a serological classification of the large number of Nag vibrios collected during the progress of the enquiry 455 strains of water vibrios and 185 Nag case strains were tested against 20 sera, including 5 sera of Gardener & Venkataraman's Groups II to VI and 15 other sera raised from local strains. With this range of sera only 182 strains (127 water vibrios and 55 Nag cases vibrios) could be grouped. Several of these groups are very small, including only a few strains. There were only 6 of any size viz., (1) group III-14 & 3 ; (2) G.W.V. 37/2-11 & 10 ; (3) G.W.V. 37/4-25 & 5 ; (4) G.W.V. 37/6-12 & 9 ; (5) G.W.V. 37/7-20 & 11 ; (6) G.W.V. 37/16-14 & 3. It will be seen that each group includes both water vibrios and Nag case strains.

4. CHOLERA CARRIER INVESTIGATION IN MADURA.

The cholera carrier survey was continued by the Health Officer, Madura City. As has already been mentioned, this survey is financed by the Municipality but the Institute continued to watch its progress with interest and helped with advice and guidance. There was no cholera in the city throughout the year and the results can only be taken as an index of the conditions in an inter-epidemic period. During the year specimens of stools from 6,301 residents in three outlying divisions were examined and vibrios isolated from 130 specimens. None were agglutinable by Group I 'O' serum. Their distribution in the Heiberg scheme was as follows :—

I - 40,	II : 30,	III - 45,	IV - 8,	V - 6	VI - 1.
30.7%	23.7%	34.5%	6.1%	4.3%	0.7%

These areas were chosen because they were not served by the city's protected water supply system and the conditions in one of the areas were very much like those in any rural community. During the course of the investigations, however, a partially protected water supply has been introduced in some of these areas.

Certain typical wells and other sources of water in these areas were also periodically examined for the presence for vibrios. 126 samples were examined and 113 were positive for vibrios. Of 388 strains of vibrios

tested, none were agglutinable. Their distribution in Heiberg scheme was :—

I - 141	II - 175,	III - 27,	IV - 23	V - 8	VI - 14.
36.3%	45%	7%	5.9%	2.0%	3.7%

The relatively greater preponderance of group III vibrios in human stools than in water, noticed early in the investigation seems to hold still.

7. Cholera (fly) transmission enquiry by Dr. M. B. Soparkar at the King Institute, Guindy, Madras.

Further work to study the role of the common house-fly in the spread of cholera was carried out and experiments were made to determine the length of time during which the cholera vibrio remains alive in the body of the house-fly after its ingestion. Laboratory-bred flies were employed. They were fed with a mixture of sterile milk and a suspension of young cholera culture to which, in some experiments, a few drops of Indian ink were added as an indicator. Altogether 22 experiments have been made up-to now in the course of which 1,058 flies have been fed, in thirteen experiments by the open watch glass method, together with an indicator—mass feeding—and in nine experiments by the capillary pipette method—individual feeding. Five hundred and twenty-two flies were dissected at intervals ranging from 2 hours to 9 days and their crops and intestinal contents were examined culturally for the presence of cholera. In only 22 instances, cholera vibrios could be isolated, from the flies up-to 8 hours after feeding and in only one more instance up-to 24 hours. Beyond this period no living cholera vibrios could be isolated from the fed flies.

The results are summarised in table I.

Cholera vibrios in fly dejecta.

Another method of study employed was to ascertain the length of time, after ingestion of food containing cholera vibrios, during which each fly continues to void cholera-vibrios in its dejecta. Individual flies were fed on a mixture of milk and a suspension of young cholera culture and were distributed singly in sterile tubes which were changed at stated periods and their vomit and faecal spots in the tubes were examined by cultural methods at intervals varying from one hour to six days after feed for the presence of living cholera vibrios.

Cholera vibrios were isolated from the faecal and vomit spots of the fly in a large number of cases (in 30 out of 72) during the first 8 hours after feed and in 2 out of 12 instances in 12 hours and in only one instance out of 12 in 24 hours. Beyond this period up-to 6 days, in no instance out of 36 flies examined was cholera vibrios isolated. It may be mentioned that in these experiments the fly drops were examined when freshly deposited and while the majority of them were still wet, so that the negative results obtained after 24 hours cannot be ascribed to the effects of desiccation which were found to be lethal for the cholera vibrio as will be seen from another series of experiments noted below.

The results are summarised in table II.

Effect of desiccation.

In order to study if the vomit or faecal drops of the fly would remain infective after their deposition by the fly on a dry surface, several

experiments were made to study the effect of desiccation on cholera vibrios. A mixture of milk and a suspension of cholera vibrios was distributed in drops of the size of the vomit or faecal drops of the fly in several tubes which were left at room temperature and the presence of living vibrios was tested at regular intervals by cultural methods. Out of 174 tubes examined at intervals varying from one to twenty-four hours, cholera vibrios were isolated in 36 out of 46 instances during *the first hour only*. At the end of one and a half hours and beyond, no living vibrios could be isolated from the dry drops in any of the 128 tubes examined, even though in the original milk mixture they were found alive for 12 days after which the milk became clotted and the cholera vibrios were found all dead, indicating that under the conditions of the experiment, cholera vibrios are rapidly killed off by desiccation.

The above results confirm the previous findings and show that the cholera vibrios when ingested by the common house-fly at a single feed are either rapidly excreted or are destroyed in the body of the fly.

A BACTERICIDAL PRINCIPLE.

In the report for the previous year attention was drawn to the possible correlation between the finding of the early disappearance of the cholera vibrios from the gut of the fly and another finding that an extract made from the abdomen of the fly when mixed with a suspension of a young culture of cholera vibrios had a bactericidal effect upon the vibrios—0.5 ml. of the extract destroying five million vibrios in about an hour or two—suggesting the presence of a bactericidal principle in the body of the fly. Attempts were made to study the nature of the principle.

As large quantities of the extract were needed for this study, the extracts were made from the whole fly caught in nature, care being taken to prevent autolytic changes taking place before extraction. This point was found to be of special importance. These extracts were found to have the same bactericidal effect on cholera vibrios as extracts made from the abdomen which method entailed dissection and was time-consuming.

Attempts were first made to see if the normal products of metabolism present in the fly were responsible for the bactericidal action. Analysis of the extract showed, among other things, the presence of urea, salts of ammonia and reducing sugars. These products, particularly urea and ammonium salts, when tested upon cholera vibrios in relatively high concentrations were found to have a bactericidal action but the proportions of these revealed by analysis of the extracts were so small that the bactericidal action could not be attributed solely to these products. A mixture containing nearly double the quantities of ammonia and urea and 2% sugar found in the fly extract, was found to be inactive even after 48 hour contact.

As extracts made from flies caught in nature were heavily contaminated with extraneous organisms, their bactericidal property could not be tested without previous sterilisation. It was found that when the extract was heated to 100 to 115° C, a thick precipitate, for the most part of coagulable proteins, was thrown down leaving a clear supernatant liquid. On testing, it was found that the bactericidal principle was present in the supernatant liquid but not in the precipitate.

Extraction with different solvents and separation of the constituents showed that the principle was soluble in water but insoluble in

acetone and ether, and that it acted quantitatively. The fact that the extract, after heating to 115°C for 20 minutes still possessed bactericidal power showed that the action was not due to an enzyme. Its resistance to heat and the results of other experiments did not support the view that the principle was of the nature of a bacteriophage. Further attempts at purification were made by precipitation with phosphotungstic acid. The clear filtrate obtained after appropriate treatment and complete removal of phosphotungstates and precipitating reagents such as barium, sulphuric acid, carbon dioxide and sulphates was found to have the bactericidal power while the precipitate was more or less devoid of it, indicating that the principle was not present in any of the substances precipitated by phosphotungstic acid such as proteins and some of their degradation products like proteoses, peptones and polypeptides, some of the amino acids such as arginine, lysine, histidine and cystine, and more particularly the amines and ammonia. Comparative tests and analysis of the original extracts and its phosphotungstic acid filtrate showed that the ammonium content of the filtrate was about one-thirtieth that of the original extract and yet both these were actively bactericidal. This point is of some importance as tests with salts of ammonia and ethylamine and methylamine showed that they possess bactericidal action on cholera vibrios.

Although treatment with phosphotungstic acid is a recognized method of fractionation in biochemical investigations, in order to remove any doubt as to the possibility of a change having been introduced in the original principle by the chemical treatment to which the extract was subjected, recourse was had to a purely physical method of fractionation viz., dialysis, which was carried out at a temperature below 8° C and which was calculated to introduce the least change in the original substance and would enable it to be recovered in its natural state.

These experiments showed that the bactericidal substance was of a crystalloid nature and was found to diffuse out in the dialysate. Complete diffusion of this crystalloid substance took about 8 days in the course of which about 5 litres of the dialysate was collected. Tests carried out on the dialysate as well as the non-dialysable residue in the bag showed that the bactericidal substance was present in the dialysate and was absent from the residue.

Another interesting observation that came to light in the course of these investigations was that when the original extract was filtered through a collodion gradacol membrane with a pore diameter of .6m or .4m specially prepared for virus studies, it was found to be only feebly bactericidal. Further interesting observations have been made in this connection which throw light on the possible nature of the bactericidal substance but they are yet incomplete.

No difference in the bactericidal effect of the extract was noted between freshly isolated vibrios and old laboratory cultures and the extract showed similar lethal effect when different non-agglutinable water vibrios were tried as test organisms. When the extract was tried on other organisms, such as *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. dysenteriae*, Flexner and Shiga, *B. coli* and *Staphylococci*, it did not appear to have the same bactericidal effect as upon cholera. The extract appeared thus to have a selective action on vibrios.

It has not been possible so far to isolate the bactericidal substance in a pure state although it has been separated in a partially pure form and

many of its characters have been studied. In order to see whether bactericidal principle is identical with allantoin isolated by Robinson and found to be present in the larva of the blow fly (Lucilia sericata) used in maggot therapy, tests with a solution of allantoin recommended by Kaplan for the treatment of infected wounds were repeated against cholera vibrios. Absence of bactericidal action in this solution and other characteristics showed that the bactericidal substance present in the common house-fly was not identical with allantoin.

Further work is necessary before the precise nature of this vibricidal substance in the body of the fly can be determined.

Summary of cholera transmission experiments with flies.

Table 1.

Examination by dissection for cholera vibrios.

Interval after feed.	Number of flies dissected.	Number positive.
2 hours	12	7
4 hours	3	1
4½ hours	2	2
5 hours	3	1
5½ hours	9	7
6 hours	1	nil
7 hours	6	nil
8 hours	9	4
10 hours	1	nil
18 hours	2	nil
20 hours	1	nil
22 hours	1	nil
24 hours	101	1
28 hours	10	nil
2 days	75	nil
3 days	55	nil
4 days	63	nil
5 days	62	nil
6 days	50	nil
7 days	51	nil
8 days	3	nil
9 days	2	nil
	522	23

Table 2.

Examination of fly dejecta for cholera vibrios.

No. of flies fed	No. of flies examined	Interval after feed	Number positive	Number negative
12	12	1 hour	2	10
12	12	2 hours	5	7
12	12	3 hours	6	6
12	12	4 hours	5	7
12	12	6 hours	8	4
12	12	8 hours	4	8
6	6	10 hours	–	6
12	12	12 hours	2	10
6	6	16 hours	–	6
6	6	20 hours	–	6
12	12	24 hours	1	11
12	12	48 hours	–	12
12	9	72 hours	–	9
12	8	96 hours	–	8
12	5	120 hours	–	5
12	2	144 hours	–	2

8. Investigation on cholera toxin and immunity in cholera by Dr. H. Ghosh at the Indian Institute for Medical Research, Calcutta.

Investigation was undertaken with a view to obtain positive data as to the quality and quantity of the chemical constituents such as sodium chloride, alkaline bases, carbonates, ammonia, protein, non-protein nitrogen, phosphorus, sulphur, etc. eliminated with enormous quantity of liquid stool passed by cholera patients

It was thought that some data might be obtained regarding the formation of cholera toxin in the intestine and the relationship between the elimination of some of the chemical constituents of the tissues and the clinical symptoms of cholera such as suppression of urine, acidosis and vaso-motor paralysis. In the following experiments attempts were made to estimate the quantity of the following chemical substances of cholera stool—viz. total solid, total alkali, total chlorides, total protein, non-protein nitrogen, ammonia nitrogen, phosphorus, sulphur, carbonates and occult blood. Stools of cases positively diagnosed by bacteriological finding as cholera were taken into consideration. The stools were collected from the cholera ward of the Chittaranjan Hospital during the last epidemic in Calcutta extending over the months of May, June, July, August and September.

Experiments.

All the experiments were made with fresh samples of stools. The stools were collected in a wide mouthed bottle containing toluene.

Total solid : 20 c.c of faeces were taken in weighed evaporating dish and dried on a water bath when completley dried. The basin was kept in a desiccator and weighed again.

Total alkali : This was determined by titrating 10 c.c. of faeces with a decinormal hydrochloric acid solution using methylorange as indicator.

Total chlorides : Whitehorn's (1921) method was applied for the estimation of chloride. The protein was precipitated from stool by tungstic acid. The chloride was then precipitated from the stool filtrate by means of silver nitrate solution in the presence of nitric acid and the excess of silver titrated with standard thiocyanate solution using Ferric ammonium sulphate as an indicator.

Total protein : Total protein was estimated by Kjeldahl method.

Non-protein nitrogen : Non-protein nitrogen was determined in a portion of the protein free stool filtrate by a micro-Kjeldahl method using a sulphuric and phosphoric acid mixture for the digestion and the ammonia formed was estimated calorimetrically after direct nesslerization of the digested mixture.

Total phosphorus : Total phosphorus of stool was determined by Whitehorn's modification of the Bell-Doisy procedure. (1924-25)

Procedure : 1 c.c of stool was taken in a hard glass test tube. 1 c.c. of concentrated sulphuric acid and 1 c.c. of concentrated nitric acid and a glass bead were introduced into the test tube. The mixture was digested over the naked flame of a micro-burner for about 10 minutes. The heating was stopped when the tube was nearly filled with fumes and allowed to cool for about three minutes. 2 c.c. of a 20 per cent. solution of sodium sulphate and another glass bead were added. The mixture was again heated as before for about two minutes. The heating was stopped as soon as the white fumes appeared. After cooling a minute or two 5 c.c. of distilled water were added and the tube was cooled in cold water. In a similar tube 5 c.c. of standard phosphate containing 0.01 mg. of phosphorus per cubic centimeter were taken and 1 c.c. of concentrated sulphuric acid was added and cooled.

To each tube 2 c.c. of acid molybdate solution (5 per cent. ammonium molybdate in 2N sulphuric acid), 2 c.c. (20 per cent.) sodium sulphite and 1 c.c. (2 per cent.) of hydroquinone solution were added in the order given, shaking after each addition. The volume was then made up to 15 c.c. with water, stoppered with rubber cork and inverted twice. The tubes were set in a boiling water bath for 10 minutes and then cooled at the tap. The colors developed were compared in a calorimeter.

Estimation of sulphur—The method adopted was that of Benedict (1909).

To 10 c.c. of stool in a porcelain evaporating dish, 5 c.c. of Benedict's total sulphur reagent were added. The mixture was evaporated to dryness on a water bath. The evaporating dish was then heated carefully until the entire residue is blackened and then finally heated to redness for about 10 minutes after the black residue which first fused became dry. The flame was then removed and the dish was allowed to cool. 10 to 20 c.c. of dilute hydrochloric acid were added and the dish was warmed gently until the contents completely dissolved and a clear sparkling solution obtained. The solution was washed quantitatively into an Erlenmeyer flask and diluted to about 100 c.c. with distilled water. 10 c.c. of a 5 per cent. solution of barium chloride were added drop by drop. At the end of an hour or later the mixture was filtered through a Gooch crucible which had previously been weighed after ignition and cooling in a desiccator. The precipitate was

washed with about 200 c.c. of water. The crucible and its contents were then dried, ignited, cooled and weighed.

Ammonia : 10 c.c. of stool was shaken with powder which removed the ammonia from the solution. The ammonia was set free from the silicate by treatment with alkali solution. This was then nesslerized and compared with a standard ammonia solution treated in the same way.

Estimation of carbonate : The carbonate present in stool was decomposed by the addition of hydrochloric acid and the carbondioxide evolved therefrom was passed through a baryta solution of known strength by means of carbondioxide free air aspiration. After the reaction, excess of baryta was titrated against a standard acid. Carbonate was then calculated in terms of carbondioxide.

The presence or absence of blood in stools was detected by Benzidine reaction.

Thirty-two samples of cholera stools have been analysed. The stools in all cases were highly alkaline. The average alkalinity has been found to be nearly equivalent to 50.95 c.c. of N/10 Hcl per 100 c.c. of stool. The average percentage of sodium chloride was found to be 442.87 mg. protein, non-protein nitrogen, ammonia, phosphorus, sulphur, and carbonate contents per 100 c.c. of stool were found to be 9.41 gm. 36.8 mg., 5.25 mg., 56.57 mg., 14.12 mg. and 0.081 gm. respectively. The percentage of total solid material has been found to be 1.94 gm. The tests for occult blood were positive in all the samples of stools examined. The sp. gr. of blood of the patients varied from 1059 to 1066.

DIFFERENT METHODS OF OBTAINING THE ENDOTOXIN OF *V.cholerae.*

Method 1. Fraction of endotoxin obtained by filtering and an emulsion of *V.cholerae* after repeated freezing and thawing.

Emulsion of 18 to 20 hours agar-culture of Vibrios was made in distilled water (10 c.c. for each Roux-bottle), put up in small phials and frozen and thawed alternately for 12 or 14 times. The emulsion was then filtered through candle L_2.

Method 2. Trichlor-acetic acid soluble fraction, obtained after digestion of emulsion of *V.cholerae* by trypsin.

18 to 20 hours agar-culture was twice washed with normal saline solution in centrifugal machine. The deposit of vibrios was then emulsified in Soerensen phosphate buffer solution having a pH of 8.4 and then digested by trypsin under toluol at 37° C for 2 days. The emulsion was then treated with equal quantity of N/2 Trichlor-acetic acid and kept in the refrigerator for 2 hours. Afterwards it was centrifugalised at high speed and the supernatant fluid separated from the residue. The fluid was then dialysed in parchment bag until it was completely neutral. It was ultimately filtered through L_2 candle and kept in refrigerator under toluol.

Chemical examination :—It contained no protein, as both Millon and Biuret reactions were negative, while it contained carbohydrate as Molisch reaction was strongly positive.

Method 3. Endotoxin fraction obtained from an emulsion of *V. cholerae* allowed to autolyse in distilled water.

18 to 20 hours agar-culture was taken in emulsion in distilled water (10 c.c. for each Roux-bottle). This emulsion was further diluted 1 in 3 or 1 in 4 in distilled water and kept in the refrigerator under toluol. This emulsion became homogeneous and opalescent after about 4 weeks. For experiments both unfiltered emulsion and the filtered (through L_2 candle) were used. The vibrios in unfiltered emulsion were all found to be dead after keeping in the refrigerator for about 2 months, as revealed by repeated culture.

Method 4. Emulsion of *V.cholerae* in normal saline solution.

18 to 20 hours agar-culture emulsion in normal saline solution, kept in refrigerator under control.

Method 5. Emulsion of *V.cholerae* in a solution, having a pH 8.4 made with equal parts of Na_2CO_3 and Na_2HPO_4.

18 to 20 hours agar-culture emulsion made in the above solution and then kept in the refrigerator under toluol.

For the preparation of above toxins only recently isolated strains, both agglutinating and non-agglutinating to pure Inaba "O"—serum were used for a comparative study. Before use, each strain of vibrio was plated several times to ensure purity of the strains used for experiment.

The following strains of *V.cholerae* were used :—

7	..	..	.. non-agglutinating type.
16/2	..	..	.. non-agglutinating type.
12/2	..	..	.. agglutinating type.

Experimental results.

In experiment rabbits were found to be more sensitive to the toxins than any other laboratory animals. So all the experiments were tried in rabbits and the intravenous route was found to be the most convenient one. The results are shown in table 1.

Table I.

No. of method of preparing cholera toxin.	No. of strain of *V. cholerae* used for preparing toxin.	Dose used.	No. of animals used and of animals which died.	Remarks.
1	7	0.1.c.c.	3/10	The animals died with symptoms of dyspnoea and spasm within ½ to 2 hours after injection.
"	"	0.2.c.c.	0/3	
"	"	0.5.c.c.	0/1	
1	16/2	0.15.c.c.	1/9	The animals died with symptoms of dyspnoea and spasm within ½ to 2 hours after injection.
"	"	0.2.c.c.	0/4	—
"	"	0.3.c.c.	0/1	—
"	"	0.5.c.c.	0/1	—
1	12/2	0.2.c.c.	0/2	—
"	"	0.5.c.c.	0/7	—
"	"	0.6.c.c.	0/1	—
2	7	2 c.c.	2/3	The animals died with symptoms of dyspnoea and spasm within ½ to 2 hours after injection.
2	12/2	1.5.c.c.	2/3	The animals died with symptoms of dyspnoea and spasm within ½ to 2 hours after injection.
3	16/2	0.5.c.c.	1/2	The animals died with symptoms of dyspnoea and spasm within ½ to 2 hours after injection.
"	"	0.75.c.c.	0/1	—
"	"	1 c.c.	0/1	—
"	"	2 c.c.	0/1	—
3	12/2	1 c.c.	1/2	The animals died with symptoms of dyspnoea and spasm within ½ to 2 hours after injection.

* The denominator indicates the number of animals used and the numerators the number of animals which died.

It will be noted from table I that the effect of toxin on animals was different in different animals. Some of the rabbits were found to have died within a very short time from ½ to 2 hours after injection with signs of dyspnoea and general spasm, while others could resist a much higher dose. It was, therefore, found impossible to find out a minimum lethal dose of any of the toxins prepared according to the above methods.

Autopsy of the animals which died within a very short time after injection with symptoms of dyspnoea and spasm revealed a slight congestion of the mesentric veins. In a few cases the animals passed semi-solid stools before death.

From the anomalous result of the action of toxin of vibrios it seemed that there existed a distinct sensitiveness to the toxins of *V.cholerae* in some of the animals. Attempts were then made to select out the sensitive group of animals to cholera toxins.

Cutaneous sensitiveness to toxins.

A number of experiments was made in order to find out whether there existed any relation between the intracutaneous reaction and sensitiveness of the animal to cholera toxins.

Preparation of toxin for intracutaneous test.

20 hours, agar-culture was made into an emulsion with distilled water and kept in the refrigerator under toluol for 3 months. Before intracutaneous inoculation this stock emulsion was so diluted in normal saline solution that 1 c.c. of the diluted emulsion contained about 2.5 mg. of dried vibrios. 0.1 c.c. of this diluted toxin was used for intracutaneous test.

Result of intracutaneous inoculation.

The reactions which were obtained could be classified into the three following groups.

1. No swelling or slight swelling at the site of inoculation with slight redness, appearing within 3 hours after injection but disappearing within 24 hours. This was taken as a negative reaction.

2. Slight swelling and redness, persisting even after 24 hours. This was also considered as a negative reaction.

3. Swelling of about 3/4th of an inch in diameter appearing within 3 hours accompanied by a redness distributed all over the swollen area and becoming prominent within 24 hours. This was considered as a positive reaction.

It was noticed that most of the rabbits of 800 g. or below gave negative reactions and most of the rabbits showing positive reactions were of higher weight.

Results of intravenous injections of cholera toxins to the skin-tested animals.

Cutaneous test was made on 12 rabbits. 4 of them showed positive reaction and when intravenous injection of toxin was given in doses varying from 0.1 c.c. to 1 c.c. all these 4 animals died with symptoms of dyspnoea and spasm within ½ to 2 hours after injection. The remaining 8 survived even after injection of higher doses of toxin. The results of the experiment are shown in table 2.

Table 2.

Weight of Rabbits	* SKIN REACTION				No. of Method of preparing Cholera toxin.	No. of strain of *V. cholerae* used for preparing toxin	Dose	Result of inoculation	Remarks
	Toxin from non-agglutinating vibrion		Toxin from agglutinating vibrion						
	3hr.	24hr.	3hr.	24hr.					
1410 g.	Sr	Sr	r	r	1	16/2	0.15 c.c.	†	Died within 2 hours of dyspnoea and spasm.
755 g.	—	—	—	—	1	16/2	0.15 c.c.	—	Did not die even after another injection of 0.15 c.c. after 24 hrs.
760 g.	r	r	r	r	1	16/2	0.15 c.c.	—	No reaction
505 g.	s	—	s	—	1	16/2	0.15 c.c.	—	Did not die even after another 2 injections of 0.15 c.c. and 0.3 c.c. at 12 hrs. interval.
890 g.	Sr	Sr	Sr	r	1	7	0.2 c.c.	†	Died within 2 hrs. of dyspnoea and spasm.
580 g.	—	r	—	r	1	7	0.2 c.c.	—	Did not die even after another injection of 0.2 c.c. after 24 hrs.
770 g.	Sr	Sr	Sr	Sr	3 unfiltered	16/2	0.5 c.c.	†	Died within ½ hr. of dyspnoea and spasm.
740 g.	s	—	s	—	3 unfiltered	16/2	0.75 c.c.	—	No reaction
1065 g.	—	—	—	—	3 unfiltered	16/2	2 c.c.	—	No reaction
645 g.	—	—	—	—	3 unfiltered	16/2	1 c.c.	—	No reaction
690 g.	Sr	Sr	Sr	Sr	5	12/2	1 c.c.	†	Died within 2 hrs. of dyspnoea and spasm.
640 g.	—	—	—	—	5	12/2	1 c.c.	—	No reaction

* S = swelling of about ¾th of an inch in diameter.
s = swelling of about ¼th of an inch in diameter.
R = redness all over the swollen area.
r = slight redness at the point of injection.
† = Dead.
— = No reaction.

From the above table the skin-positive rabbits seemed to be distinctly sensitive to the toxins, while the skin-negative ones were very resistant. The number of experiments, carried out is not of course, adequate enough to reach to a definite conclusion. Further experiments to corroborate the above result are being carried on.

EFFECT OF REPEATED INJECTIONS OF TOXIN IN SKIN-NEGATIVE RABBITS.

A few rabbits which showed negative skin reaction were given repeated intravenous injections of toxin. The result is shown in table 3.

Table 3.

Weight of rabbits.	No. of method of preparing cholera Toxin.	No. of strain of *V. cholerae* used for preparing toxin.	Date and dose of inoculation.	Date and dose of inoculation.	Date and dose of inoculation.	Date and dose of inoculation.
1390 g.	1	7	11-6-38 at 12 P. M. 1.5 c.c.	12-6-38 at 12 P.M. 0.5 c.c.	13-6-38 Died previous night.	
1180 g.	1	12/2	11-6-38 at 12 P. M. 1.5 c.c.	12-6-38 at 12 P.M. 0.5 c.c.	13-6-38 at 12 P.M. 0.5 c.c.	14-6-38 at 12 P.M. 0.1 c.c. Died at 5 P.M
645 g.	3 unfiltered	16/2	28-10-38 at 11 A.M. 1 c.c.	29-10-38 at 11 A.M. & 3 P.M. 1 c.c. each.	30-10-38 Found dead.	

SYMPTOMS IN ANIMALS AFTER REPEATED INJECTIONS OF TOXIN.

There was decided manifestation of toxic symptoms with higher and repeated doses of toxin. Most of the animals thus injected exhibited signs of sickness, remained absolutely quiet, passed semi-solid followed by liquid stools and frequently drank water. After repeating the injection 1 to 4 times at intervals of about 12 to 24 hours the animal died within 48 to 72 hours.

Autopsy of these animals revealed liquid and occasionally colourless stool in the small intestine. The small intestines were congested. The descending colon and rectum were completely empty. The kidneys were enlarged and highly congested. The suprarenal bodies were also very congested. In a few cases peritoneal effusion was present.

ROLE OF ASSOCIATED ORGANISMS IN ENHANCING THE TOXICITY OF THE CHOLERA TOXIN.

Sanarelli showed that when a rabbit was injected with living *V. cholerae* followed after 24 hours by a filtered broth-culture of B. coli, the animal used to succumb and autopsy revealed acute congestion of the small intestine and peritoneal effusion. When, however, the same dose of living

V.cholerae or the toxin of B. coli was injected singly the animals used to survive.

With a view to find out whether toxin of associated intestinal micro-organism could influence the toxicity of cholera toxin the following experiments were carried out.

EXPERIMENT.

Each skin-negative rabbit was injected with 0.1 c.c. to 0.6 c.c. of endotoxin of *V.cholerae* followed after 4 hours by 0.1 c.c. to 0.2 c.c. of filtered broth culture of B. coli. The results are shown in table 4.

Table 4.

Weight of skin-negative rabbit.	No. of method of preparing cholera toxin.	No. of strains of *V.cholerae* used for preparing toxin.	Dose of cholera toxin.	Dose of B. coli toxin.	Result.
1300 g.	1	16/2	0.2 c.c.	0.2 c.c.	No reaction
625 g.	1	16/2	0.2 c.c.	0.2 c.c.	No reaction
530 g.	1	16/2	0.2 c.c.	0.2 c.c.	No reaction
1615 g.	1	12/2	0.5 c.c.	0.2 c.c.	No reaction
1075 g.	1	12/2	0.5 c.c.	0.2 c.c.	No reaction
535 g.	1	12/2	0.5 c.c.	0.2 c.c.	No reaction

From the table 4 it will be noticed that there was no enhancement of toxicity of cholera toxin when associated with the filtered toxin of B.coli in skin-negative animals.

MALARIA

9. The Malaria Institute of India, Kasauli.

DESIGNATION.

The name of the organisation has been changed from 'Malaria Survey of India' to 'Malaria Institute of India', with effect from 1st April, 1938.

STAFF.

(i) Lt.-Col. G. Covell, I.M.S., Director. (ii) Major M. K. Afridi, I.M.S., Assistant Director. (iii) Major Jaswant Singh, I.M.S., Research Officer, from July 14th onwards. (iv) Dr. I. M. Puri, Entomologist, was on leave ex-India for eight months from the 25th of April. (v) Rai Sahib

Dr. Khazan Chand, P.C.M.S. held the post of Assistant to Director except when he was on leave on average pay from 1st April to the 11th May, 1938.

TOURS.

(i) Lt.-Col. Covell toured in South India from 14th April to 6th May. He visited the areas selected for the Madras Presidency rural antimalaria scheme, and inspected the work of the research unit of the institute located in the Wynaad. (ii) Lt.-Col. Covell toured in Bengal and Orissa from 6th to 16th September. He visited the areas proposed for the Bengal rural antimalaria scheme, and a number of malarious areas in Orissa. (iii) Lt.-Col. Covell made a second tour in South India from 3rd to 18th October. He visited a number of centres in Coorg, inspected the work of the research unit in the Wynaad, and investigated malarial conditions in Bangalore. (iv) Major Afridi visited the U. P. Terai from 6th to 9th April to make arrangements for a research unit of the Institute to commence work there, and to advise on the U. P. rural antimalaria scheme. He paid three subsequent visits to this area in August, October and November to inspect the work of the research unit. (v) Major Afridi visited Karachi on 28th September to demarcate the area round the aerodrome for mosquito control. (vi) Major Afridi visited Kutch State from 16th to 20th November, 1938 for inspection of and advice on the antimalaria measures being carried on in the state. (vii) Dr. Puri, whilst on leave in Europe made a study tour in Italy, Albania and Holland, which was financed by the Rockefeller Foundation. He also represented the Government of India at the seventh International Congress on Entomology at Berlin in August, and at the third International Congress on Malaria at Amsterdam in September.

SURVEYS.

(i) A unit consisting of a sub-assistant surgeon, a laboratory assistant and an insect collector, under the direction of Major Afridi, carried out a malaria survey of Bahrein Islands, Persian Gulf, in May and June. (ii) Major Afridi, accompanied by a sub-assistant surgeon and an insect collector, made a mosquito survey of Hog Island (Bombay) in the last week of July.

CLASSES, INSTRUCTION, ETC.

(i) The annual malaria class, held at Karnal in March and April, was attended by 20 students, 17 of whom passed the examination. (ii) Two sanitary inspectors from the N.-W. Railway were trained in practical control work from 28th March to 14th April. (iii) A number of unpaid apprentices have been trained as insect collectors at Karnal and Delhi.

PUBLICATIONS, REPORTS, ETC.

(*a*) *Records of the Malaria Survey of India.* In conformity with the change in the title of the organisation, this publication has been re-named '*Journal of the Malaria Institute of India*'. Four numbers containing 33 papers and 4 abstracts, have been published during the year. Of the 37 papers above referred to the following were the work of members of the staff :—

(1) The Malaria Survey of India. (Covell).
(2) Destruction of mosquitoes with insecticidal sprays. (Covell, Mulligan and Afridi).
(3) An apparatus for mixing larvicides. (Butt).
(4) Range of dispersion of *C. fatigans*. (Afridi and Majid).
(5) Malaria in Kutch State. (Afridi, Majid and Jaswant Singh).

(6) The extra-abdominal spleen in monkey malaria. (Afridi).

(7) Malaria in Bahrein Islands. (Afridi & Majid).

Health Bulletins.

Published.

(1) Table for the identification of adult anophelines. (Christophers, Sinton, Covell & Barraud, revised by Puri), 3rd Ed.

(2) Table for identification of anopheline larvae (Puri), 3rd Ed.

(3) Man-made malaria in India. (Sinton & Raja Ram), 2nd Ed.

(4) Table for the identification of fish. (Hora & Mukerji), 2nd Ed.

(5) Prevention of malaria incidental to engineering construction. (Mulligan and Afridi).

In Press.

(6) Practical entomological course for students of malariology. (Barraud, revised by Puri), 2nd Ed.

(7) Suggestions regarding prevention of spread of yellow fever to India by air traffic (Sinton), 2nd Ed.

(8) What malaria costs India (Sinton).

Other publications.

(1) Monthly reports on antimalaria operations in Delhi (Covell, Mulligan & Afridi), July 1936 to June 1938.

(2) Chart showing action of quinine and synthetic drugs on malaria (Based on League of Nations Malaria Commission's 4th General Report).

(3) Antimalaria operations in Delhi (Afridi) *Proc. Nat. Inst. Sc. India*, IV, 2, pp. 201-2.

MISCELLANEOUS REPORTS AND NOTES.

These have been submitted on (1) control measures in Delhi, (2) treatment of malaria with antimony compounds, (3) modern treatment of malaria, (4) malaria control in U. P. Terai, (5) malaria control in Coorg, (6) malaria control in Savantvadi State, (7) malaria in Orissa, (8) malaria in Bangalore, (9) use of atebrin in India, (10) malaria in Behrein Islands, (11) *Aedes* survey of Hog Island, (12) spring mosquito nuisance in Bhatinda, (13) malaria parasites in Portuguese East Africa, (14) mesh of wire gauze for house-fly campaigns, (15) effect of clover on malaria, (16) effect of *tulsi* plant on malaria, (17) control of mosquito breeding by herbage packing, (18) spraying of dwellings with pyrethrum insecticides to kill adult mosquitoes.

SCIENTIFIC MATERIAL SENT TO OTHER WORKERS.

Monkey malaria strains have been sent to various workers in India, England and America. Mounted specimens of mosquitoes, slides showing specimens of occysts and sporozoites, and *Gambusia* fish have been sent to various workers in India.

Mosquito catching tubes as designed by Major Jaswant Singh, I.M.S. were sent to the A.D.P.H. (Malariology), U. P., Lucknow.

ROUTINE ENTOMOLOGICAL WORK.

Expert identification of mosquitoes and sandflies has been carried out as in former years, numerous collections having been received from workers in all parts of India. The collection and preparation of large numbers of mosquitoes for instructional purposes, demonstration, study and exchange have been continued.

TESTING OF LARVICIDES.

A number of these has been tested under field conditions.

TESTING OF INSECTICIDES.

A number of proprietary insecticides has been tested in the experimental chamber. Those containing pyrethrum extracts continue to be the most effective. A sample of dried pyrethrum flowers grown in Kulu and soaked in kerosene oil for 72 hours gave very encouraging results. It is confidently expected that it will eventually be possible to produce a pyrethrum insecticide at a very cheap price in this country.

ANTIMALARIA OPERATIONS IN DELHI.

These have again been supervised by officers of this Institute. Close contact has been maintained with the Central P.W.D. in carrying out certain engineering works remaining over from the 1937-38 programme. Recurring control measures have been carried out on the same lines as in the two previous years. The most important advance has been the employment of various forms of oil booms in the Western Jumna Canal and Najafgarh Cut. The reduction in malaria incidence has been marked, but it is recognized that the control measures have been greatly aided by the deficiency of monsoon rainfall.

THE LIBRARY.

This has been extended by purchase and exchange, and various publications have been sent on loan to workers in other parts of India.

THE MUSEUM.

Numerous photographs illustrating malaria control measures in India and elsewhere have been added.

THE FIELD STATION.

Flagstaff House, Alipore Road, Delhi has been allotted by the Government of India to accommodate the field station of the Institute. The necessary structural alterations to the building are now being carried out, and it is expected that the move from Karnal will be completed early in the new year.

FIELD RESEARCHES.

(i) *Field Station, Karnal.* Observations on the bionomics of *A. culicifacies* have been continued. A large amount of very valuable data has been accumulated and is now being analysed with a view to publication. (ii) *Bahrein Islands. A. Stephensi* was incriminated as the principal, and probably the sole local malaria carrier. (iii) *Investigations in Wynaad.* A research unit, consisting of a sub-assistant surgeon two laboratory attendants and one insect collector has been working in this area throughout the period under review. *A. fluviatilis* has been incriminated as the chief malaria carrier, and has shown a very high infection rate. Out of 1313 specimens dissected in May, 287 were found to be

infected. Every aspect of the malaria problem in this area is being investigated with the object of formulating practical control measures. (iv) *Investigations in the U. P. Terai.* A research unit, consisting of a sub-assistant surgeon and 4 insect collectors has been investigating malaria conditions at Bazpur since May. The lines of work have been similar to those employed by the Wynaad unit, namely, the study of malaria among the indigenous and immigrant population by periodical spleen and blood examinations, together with detailed observations on the bionomics of the carrier specimens of mosquito, with the object of devising and testing out practical control measures which will be applicable to foothill malaria regions throughout India. (v) *Investigations in Delhi.* Experimental spraying of houses to destroy adult mosquitoes in two villages within the urban area was continued during the past malaria season. In addition, the spraying of dwellings in 6 villages in Delhi Province was organised, the work being carried out as part of the Delhi rural antimalaria scheme. The failure of the monsoon and consequent low level of transmission throughout the Province has rendered it impossible to assess the value of this method with accuracy. But it has been shown that it is possible to apply it in rural areas in this part of India, and it is hoped that it will prove a valuable weapon in dealing with epidemics.

LABORATORY RESEARCHES.

(i) Intensive observations on the behaviour of malaria parasites have been carried out on monkeys, using the *extra-abdominal spleen technique.* The factors influencing the numerical increase and decrease of parasites in monkey malaria have been studied by this method. Observations on malarial immunity have been continued. (ii) A number of strains of *monkey malaria plasmodia* have been maintained at Kasauli and Karnal. Strains of bird malaria have also been studied at Karnal. (iii) A large series of *precipitin* tests has been carried out by Barber's technique to determine the blood preferences of mosquitoes collected in the U. P., Madras Presidency, Karnal and Delhi. This work is being continued. (iv) The efficacy of certain *drugs* said to be of value in the treatment of malaria has been tested on monkeys. None of these were found to possess any significant antimalarial value.

10. Malaria transmission enquiry by Dr. B. M. Das Gupta at the School of Tropical Medicine, Calcutta.

The enquiry has been in the hands of Dr. B. C. Basu, D.Sc., and the report is written by him.

During the period under review, a total of 3,997 laboratory bred clean female *A. annularis* (Fuliginosus) were fed on 48 gametocyte-carriers (*P. vivax* 19 ; *P. falciparum* 26 ; and *P. malariae* 3.) and immediately after feeding were exposed to 10 different combinations of temperature and relative humidity in the air conditioning cabinet. A gametocyte count, in each case, was carried out on the host just before feeding the mosquitoes. The detailed results are shown in Table I.

Table I. (*Malaria*)

Combinations of temperature and relative humidity	Percentage of infection with			Longevity of *A. annularis*
	B. T.	M. T.	Q.	
50°F— 70%	0%	0%	?	67.7% (4-19 days).
50°F— 80%	0%	0%	?	68.5% (8-20 days).
50°F— 90%	0%	6.7%	0%	72% (10-27 days).
50°F—100%	0%	0%	?	74% (6-19 days).
60°F— 50%	0%	57.7%	?	58.8% (4-19 days).
60°F— 60%	27.4%	48.7%	?	55.1% (8-13 days).
60°F— 70%	33.6%	23%	0%	66.9% (6-9 days).
60°F—100%	16.9%	23.5%	?	70.3% (8-13 days).
90°F—100%	55.5%	65.9%	?	29.7% (10-12 days).
130°F— 70%	—	—	—	0% (2-4 days).

The effect of various antimalarial drugs upon the infectivity of patients to mosquitoes is being studied in collaboration with Col. Chopra. Two reports on the subject had already been written by Col. Chopra and Dr. Basu. One has been published in the Indian Journal of Medical Research and another will appear in the December number of the Journal of Malaria Institute, India. In this connection three drugs, *viz.* 'Cilional', 'M3' and 'Prontosil' are under investigation at present.

The chemo-therapy of anti-malarial drugs in general is being studied in collaboration with and under the direction of Col. Chopra, both with human and monkey malaria. Three reports were published on the subject. The efficiency of the drugs of the sulphanamide group is under investigation at present.

Dr. Basu has described a new species of bird malaria (*P. heroni*) and published an extensive report on the subject in the Journal of Malaria Institute, India, 1938.

11. Blackwater fever enquiry; vitamin C and malaria; under Dr. K. V. Krishnan at the All-India Institute of Hygiene and Public Health, Calcutta.

With the advent of Tillman's method of estimation of vitamin C in the blood and of Harris and Ray's saturation test for vitamin C in the urine, the changes in vitamin C metabolism in health and disease have been frequently investigated. From these investigations, although it has been established that vitamin C is generally depleted from the body during pyrexial attacks, there is no information available regarding the degree of depletion of the vitamin in malaria. The present investigation was therefore undertaken to elicit the vitamin C saturation in normal and malarial subjects resident in a hyperendemic malarial village in Bengal.

Children between the ages of two and ten were alone examined. The twenty-four hourly excretion of ascorbic acid in the urine of the children was determined by the well known technique of titrating it with a

standard solution of dichlorophenolindophenol. After ascertaining the rate for three consecutive days, a test dose of ascorbic aicd (equivalent to 50 m.gms. of ascorbic acid per stone of body weight) was given and the variation in the rate of excretion of the acid during the ensuing twenty-four hours ascertained. According to the results obtained the individuals were classified as "normal", "borderline", or "subnormal".

Taking an excretion of 13 m.gms. and over as normal, 10 to 13 m.gms. as 'borderline' and less than 10 m.gms. as 'subnormal', the results obtained, with respect to the amount of ascorbic acid excreted by an individual in 24 hours, are shown below :—

Table 1.

Table showing percentage incidence of vitamin C deficiency in normal and malarial subjects studied by the twenty-four hourly ascorbic acid excretion in urine. (Average of 3 consecutive days, readings have been taken).

	Healthy persons (total 20)	Malarial cases (total 33),
Normal	80%	27.3%
Borderline	15%	9.1%
Subnormal	5%	63.3%

If, on the other hand, vitamin C deficiency was judged by the saturation test, by taking an excretion of 50 per cent. or more of the test dose within 24 hours as normal, 25 to 50 per cent. as borderline and less than 25 per cent. as subnormal, the results were found to be as follows :

Table 2.

Table showing the percentage incidence of vitamin C deficiency in normal and malarial subjects as studied by the test dose method.

	Healthy persons (total 20)	Malarial cases (total 33)
Normal	10%	6.1%
Borderline	55%	6.1%
Subnormal	35%	87.8%

From these two tables and from a detailed study of the variations in individual cases, during the different stages of the malarial paroxysm, the following conclusions have been drawn :—

(1) That the majority (65 to 95%) of healthy children between the ages of 2 and 10 show normal vitamin C saturation.

(2) That the majority (60 to 87%) of malarial patients show a condition of hypovitaminosis C.

(3) That during an acute attack of malaria there is an increased excretion of ascorbic acid in the urine and during the afebrile interval the excretion is markedly less.

(4) That in chronic malarial subjects there is a well marked sub-scurvy state which is rendered more and more intense by every subsequent attack of fever.

(5) That the degree of hypovitaminosis C is most intense in malarial subjects belonging to a low economic status.

12. Enquiry on the maxillary index of A. stephensi in Calcutta by Mr. Senior White.

The enquiry was sanctioned for a second year from April, 1938, but was temporarily suspended on May 31st, for the following reasons :—

(a) The Officer in charge proceeded on 8 months' leave as from May 11th.

(b) The work which was planned to be carried out during his absence was the collection of blood meals for precipitin tests for the origin of such bloods. The Director, Malaria Institute of India, was at the time experiencing great difficulty in obtaing a supply of dried anti-sera and the carrying out of the proposed programme of study was therefore impossible.

In consultation with the Public Health Commissioner it was therefore decided to apply to the Secretary of the Indian Research Fund for permission to suspend the enquiry until the return of the officer-in-charge from leave in January.

The report now furnished therefore applies to the five months December, 1937 to April, 1938 only. In considering it the partial December figures included in the report dated 23-12-37 should be deleted.

The captures for these five months have been as under :—

Month.	No. of houses searched.	Houses catch.		Human trap net catch.		Cattle trap net catch.		Open cattle shed catch.		Total catch.	
		○	○ +	○	○ +	○	○ +	○	○ +	○	○ +
1937 December	684	0	6	0	6	0	6	—		0	13
1938											
January	690	0	5	—		0	12	0	1	0	18
February	617	0	7	—		0	16	0	7	0	30
March	680	0	5	—		0	11	—		0	16
April	661	0	7	—		0	8	—		0	15
Total	3332	0	30	0	1	0	53	0	8	0	92
April—November, '37.	5569	2	53	0	2	1	71	0	2	0	128
Grand total.	8901	2	83	0	3	1	124	0	10	3	220
Percentage of catch.		37.7		1.4		56.4		4.5			

February shares with June the production of the largest catches. The months are completely dissimilar climatically. There is thus no evidence that there is any seasonal cause for variations in numbers captured.

The full year's figures alter the impressions recorded in the first paragraph of page 3 of last year's report. Whilst rather more than half the specimens have been taken biting cattle, over one-third have been found resting in houses. But, from the very small numbers in the human bait trap net, it would appear that presumably having bitten cattle *A. stephensi* frequently rests by day in houses. But the smallness of the total catch indicates that the preferred resting places of the species in Calcutta are unknown. In an attempt to find these attention has been paid to rat holes and the tiles of ceilings. A citronella-water mixture was sprayed into such spots to drive out any resting mosquitoes into a net. Whilst the table below shows that several species were obtained by this method, *A. stephensi* was not among them, and we are no nearer a solution of the problem.

Mosquitoes from rat holes.

Month.	*C. fatigans*		*C. tritaeniorhynchus*		*C. gelidus*		*S. fasciata*		*A. subpictus*	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Februaay	42	113	0	6	3	2	2	4	—	
March	47	104	0	4	2	1	2	1	—	
April	28	29	—		—		1	0	0	1
Total	117	246	0	10	3	2	5	5	0	1

Mosquitoes from tiled ceilings.

Month.	*C. fatigans*		*C. tritaeniorhynchus*		*C. gelidus*		*S. fasciata*		*A. subpictus*		*A. annularis*	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
February	19	111	—		—		1	1	1	3	—	
March	49	127	—		—		2	4	3	2	0	1
April	43	65	—		—		3	1	3	0	—	
Total	111	303	—		—		6	6	7	5	0	1

To April 30th a total of 115 maxillae have been counted from those caught in houses or trapped on man, 220 from those caught in a cattle shed or trapped on the cow, with the following counts :—

	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	Total	Maxillary index.
Houses	—	0	1	12	26	39	19	11	5	2	0	0	115	13.1
Cattle	—	0	0	13	43	68	57	31	6	1	0	1	220	13.3

Until the full desired number of 200 in each set is obtained, it has not been considered worth-while again to test whether the dispersion of the two series shows any difference from the significant difference found in the first report.

The total number of specimens dissected now amounts to 187, but no gut or gland infection has been obtained. Of these maxillae, only the following number have been obtained from specimens of known race, all "B" :—

Resting in houses	—	19
,, ,, cattle sheds	—	8
Caught in trap net on cow	—	36

These numbers are too small to yield any information yet regarding the maxillary index of this form, or of difference in dispersion about the mean for specimens from various sources.

Captures, from December 10th, have been given a chance to oviposit before dissection, to show whether they belonged to form "B" or form "M" of Sweet and Rao.

Of thirty batches of eggs measured, 25 are definitely form 'B'. The remaining five are apparently form 'M', but all were infertile, and four of them abnormal.

Out of 46 specimens, 41 are definitely 'B', 5 doubtfully 'M'. This work will be pursued until it is known whether form 'M' is present in Calcutta in sufficient numbers to render useless last year's maxillary counts on specimens of unknown race, or whether the proportion of 'M' in the local *stephensi* population is so low that the error introduced by a few specimens can be neglected and the old counts used.

Data regarding the habits of the common mosquitoes of Calcutta as revealed by the human and cattle trap net catches have continued to accumulate. Incidentally the numbers throw a rather unfavourable light on the efficiency of the Calcutta Corporation's mosquito control, both as regards *C. fatigans* and *Stegomyia fasciata.*

TRAP NET ♀ CATCHES 1937-38, 13 MONTHS (p.p.).

	A. subpictus.	A. vagus.	A. varuna.	A. stephensi.	A. annularis.	A. pallidus.	A. ramsayi.	A. hyrcanus.	A. barbirostris.	C. fatigans.	C. vishnui.	C. tritaeniorhynchus.	C. gelidus.	C. bitaeniorhynchus.	M. annuliferus.	Ar. obturbans.	St. fasciata.
Human trap.	58	3	1	3	1	..	..	1	..	1428	17	5	3	4	..	10	1611
Cattle trap.	367	21	..	119	20	1	1	6	1	1618	15	..	39	1	2	23	333

Permission has been sought to resume the enquiry as from February 1939, and during the next financial year,

13. Assam Medical Research Society (Malaria Section).

1. Staff.

The staff has numbered 31, inclusive of 7 sub-assistant surgeons, Public Health Department, deputed for one year's training in malariology.

One member of the staff attended the course in malariology given by the League of Nations at Singapore.

One member attended the course in malariology given by the Malaria Institute of India at Karnal.

2. Training classes.

The eight weeks' course in malariology for medical licentiates was attended by 13 candidates, all of whom received certificates of proficiency after passing the examination set at the termination of the course. Six candidates were sub-assistant surgeons of the Public Health Department; three sub-assistant surgeons of the Medical Department; one sub-assistant surgeon of the Assam Bengal Railway and three private candidates holding L.M.P. qualifications.

A four weeks' course on malaria control methods for malaria Inspectors will be given during November. Ten candidates have been selected for this course.

Inclusive of the 1938 class, the Society have now completed the training in malariology of 107 medical licentiates, of whom 63 were sub-assistant surgeons of the Medical and Public Health Departments, Government of Assam, and 44 were licentiate employees of Local Boards, Assam Bengal Railway, Assam Oil Company or Assistant Medical Officers of Tea Estates in the Province.

Seventy men have completed their training as malaria inspectors and have subsequently been placed in employment.

3. Malaria surveys.

Three new malaria survey projects were undertaken during the year in addition to the completion of the survey of an extensive bhil area commenced in 1937.

4. Malaria control projects.

In addition to six *experimental antilarval control* centres, thirteen other antilarval control projects, receiving financial assistance from Government of Assam, and where a full year's survey had been completed by the Society, have been supervised by the Society's staff. Similarly, seven tea estates that have adopted the Society's recommendations for malaria control following the completion of a full year's survey, have been visited periodically for purposes of supervision of their control activities.

5. Experimental malaria control projects.

Experiments to determine the efficiency of cold weather and pre-monsoon antilarval control have been continued during the year under review.

(*a*). *Nowgong town.* This town has a population of 11,000 and is in its third year as an experimental malaria control project. It was originally classified as "Group B" (Rice 1935) and in the cold weather of 1934-35, an attempt was made to establish antilarval control of the Kalong River, the Mori Kalong and the Pota Kalong, the only water areas incriminated

of breeding *A. minimus*, the proved vector species, during a full year's survey. Unfortunately oil was used as a larvicide and this had to be discontinued after 2 months' control activity as these water areas served as the only source of domestic water supply. Active control measures therefore were only commenced from the cold weather season of 1935-36.

The control activities have consisted of the application of 5 per cent. paris green diluted with soft stone to the three incriminated water areas during the months January to, and including, June by which period the areas are in flood or flooded. Oiled booms to prevent larval drift are placed at the highest point of control of the river.

Spleen and Parasite rates of children 2 to 10 years of age were 51.2 and 34.6 per cent. respectively in the cold weather 1935-36, had been reduced to 8.8 and 4.3 per cent. respectively in a comparable period of 1937-38.

Among a comparable number of prisoners in the Nowgong Jail the hospital admissions for malaria were 98 during 1935, 18 during 1936 and 1 during 1937.

(*b*). *Gauhati town.* Gauhati has a population of 22,000 and is now in its fourth year as an experimental project. Originally classified as "Group B" antilarval oiling of the Bharalu River, its subsidiary streams, drains and small streams in the eastern part of the town was commenced during the cold weather 1934-35. Other small streams and a bhil were subsequently incriminated of breeding *A. minimus* and were included in the control programme.

Control activities have consisted of applying oil to the river, drains and some streams from January to June and in October and November. Oiled booms, screens or guddas for the prevention of larval drift are placed in river and streams at the highest point of control. In some small streams in the east of the town oiling has had to be continued throughout the year. The bhil area is treated with a 5 per cent. paris green mixture.

Spleen and parasite rates of children 2 to 10 years of age were 21.6 and 24.0 per cent. respectively in 1934-35, had been reduced to 6.1 and 6.6 per cent. respectively in a comparable period of 1937-38.

(*c*). *North Lakhimpur town.* A town of 2,200 population, now in its fourth year as an experimental control centre. In 1933 and 1934, antilarval control measures were applied from mid-March to mid-November in accordance with recommendations previously made by other workers but little appreciable reduction in the spleen and parasite rates resulted. A resurvey started in November 1934 demonstrated prolific breeding of *A. minimus* in two streams during the cold weather when this area was classified as "Group B". Antilarval control measures by oiling commenced in February 1934 and continued until mid-June when natural control by flushing was demonstrated to be adqeuate. In subsequent years this form of antilarval control has been commenced in January. One area, where one river creates swampy conditions, has been treated with paris green during the months January to June.

In the cold weather 1934-35, before commencing cold weather and pre-monsoon antilarval control, the spleen and parasite rates among children 2 to 10 years of age were 31.6 and 46.4 per cent. respectively. In the comparable period of 1937-38 the spleen and parasite rates were 23.3 and 10.8 per cent. respectively.

(*d*). *Mangaldai town.* A town with a population of 1,800, in its fourth year as an experimental control project. During 1933 and 1934 antilarval

control measures were adopted in keeping with recommendations of other workers, i.e., larvicides were applied from mid-March to mid-November to water areas incriminated, during any period of the year, of breeding *A. minimus*. No reduction in the malaria incidence was apparent following two years of control activity on this basis; the spleen and parasite rates at this time (cold weather 1934-35) being 31.5 and 25.7 per cent. respectively.

Two dying river areas having proved on survey to breed *A. minimus* in all periods of the year, this project was classified as "Group C". Antilarval control with oil or paris green, according to water areas requiring treatment, was commenced in mid-January 1935. Living rivers are treated with larvicides until monsoon flushing is established and the dying river loops receive antilarval attention throughout the year. Other water areas are ignored.

Spleen and parasite rates of children 2 to 10 years of age which were 31.5 and 25.7 per cent. respectively in 1934-35, have been reduced to 7.8 and 2.2 per cent. respectively in 1937-38.

Among prisoners in the Mangaldai Jail the hospital admissions for malaria and for all causes respectively were 71 and 130 in 1935; 23 and 47 in 1936 and 12 and 32 in 1937.

(*e*). *Surma valley villages* (*Jagadishpur Group*). A group of villages in the Habiganj Sub-division of Sylhet having a population of approximately 2,000, and now in its third year as an experimental control project. Classified as "Group B" because no *A. minimus* larvae could be collected subsequent to the establishment of natural control by monsoon flushing, the small streams incriminated of breeding that species in the cold weather and pre-monsoon seasons have received larvicidal treatment from early January until about mid-June.

Antilarval control has consisted of the application to the stream edges of 5 per cent. paris green in soft stone diluent. At the higher-most limits of stream control oiled booms have been installed to prevent larval drift. Water areas other than streams have been ignored.

Spleen and parasite rates among children 2 to 10 years which were 48.0 and 40.1 per cent. respectively in the cold weather of 1935-36 have been reduced to 8.9 and 10.5 per cent. during a comparable period of 1937-38.

(*f*). *Brahmaputra valley villages* (*Dishpur group*). A group of villages in the Kamrup District having a population of about 1,800, and now in its third year as an experimental control centre. Originally it was classified as "Group B" for no *A. minimus* were collected from the two streams present after monsoon flushing took place. Subsequent observation has revealed small malariogenic seepages in adjacent hill areas, and in addition, approximately 370 acres of rice land was irrigated during the second year of the experiment. In these areas *A. minimus* breeding continued throughout the year, hence the classification has had to be changed to that of "Group C". Control measures have now been adopted in keeping with this change.

The two malariogenic streams have been controlled during the months January to, and including, June by the use of 5 per cent. paris green in soft stone diluent and oiled screens placed at the upper limits of control for larval drift prevention. A road culvert is being reconstructed to allow for

rapid drainage of irrigated rice land and swamp areas. Seepages are being drained to reduce the areas demanding antilarval control and an attempt is being made to establish dense shade in some of these areas. Irrigated rice land is being treated with paris green in an attempt to control this extensive area.

In the cold weather of 1935-36 spleen and parasite rates were 76.5 and 54.6 per cent. respectively. In a comparable period of 1936-37, these indices had been reduced to 43.2 and 26.6 per cent. respectively. During 1937 and 1938, the villagers, although they had previously agreed not to do so, cut the bank of one river for the irrigation of rice land. As a result of this irrigation the spleen and parasite rates were increased by the cold weather of 1937-38 to 62.8 and 38.6 per cent. respectively.

6. Entomological.

Routine examination carried out between 1st January and 30th September, 1938, have included the identification of 70,759 anopheline larvae, and 8,044 adults. Anopheline dissections numbered 2,724; *A. minimus* only being found infected or infective.

From 1st June 1931 to 30th September 1938, 840,448 anopheline larvae have been collected; and their respective breeding places and dates of collection recorded. Up to the same period 138,273 adult anophelines have been identified and 54,502 have been dissected.

7. Blood and Spleen examinations.

During the year to 30th September, 10,596 blood specimens were examined for malaria parasites and 10,709 spleens palpated for evidences of enlargement.

From 1st June 1931 to 30th September 1938, 116,315 blood specimens have been examined and the findings recorded by plasmodium species and for the presence or absence of gametocytes.

8. Treatment schemes.

The Doom Dooma cinchona-plasmoquine treatment experiment has been continued for its sixth year. In 1932-33 spleen and parasite rates of children 2 to 10 years were 56.5 and 40.7 per cent. respectively. Following treatment these indices have been reduced to 14.6 and 13.5 per cent. respectively in 1937-38. In 1936, a year of high malaria incidence, the parasite rate rose to 41.0 per cent. Many tea estates have now adopted the method of treatment on the results shown by the experiment.

Two groups of villages, one group in each valley, were selected for treatment of all proved malaria cases with reinforced cinchona febrifuge. These treatment schemes were financed jointly by Government of Assam and the Local Board concerned.

9. Researches.

A rapid method for the estimation of the arsenious oxide content of paris green has been developed. Particulars of the method were published in the Jour. Mal. Inst. of Ind., Vol. 1, No. 2, June 1938.

Studies of the cold weather bionomics of *A. minimus* in the plains of Assam have confirmed the previous report (Rice and Mohan 1936).

Methods for the prevention of larval drift have been improved and have been shown to be effective.

The experimental cold weather and pre-monsoon malaria control projects were continued.

10. PROVINCIAL MALARIA CONTROL.

In co-operation with the Public Health Department and the Provincial Advisory Malaria Committee, recommendations and supervision have been maintained for antilarval and treatment schemes where surveys had previously been completed by the Society's staff.

NUTRITION.

14. Nutrition researches under Dr. W. R. Aykroyd, at Coonoor.

During the year the work carried out has in general followed the scheme outlined by the Nutrition Advisory Committee, I.R.F.A., at its various meetings. As stated in the Report of the Committee held in November 1937, "the main objective should be to obtain a clear cut picture of the problem of nutrition in India and to discover methods for improving the diet of the population which are feasible under present economic conditions. Future work should include basic nutrition research, further surveys of diet and state of nutrition in parts of the country in which such investigations have not hitherto been carried out, feeding experiments on infants and school children, the study of methods of detecting malnutrition, the study of the relation between various diseases and malnutrition, collaboration with agricultural authorities, and the preparation of suitable educational material."

SYSTEMATIC SURVEY OF INDIAN FOODSTUFFS.

A number of chemical analyses of foodstuffs have been carried out to fill up gaps in published tables. The second edition of Health Bulletin No. 23 will contain analyses of some 250 foodstuffs, 50 more than the first edition. Hindustani equivalents for the names of most of the foodstuffs have been included in an appendix, and a section on infant feeding has been included. Samples of almost all common Indian foodstuffs have now been studied and this part of the survey may be regarded as reasonably complete. The data obtained have been widely used all over India by workers carrying out diet and economic surveys, by medical and health officers, superintendents of institutions, as also by the general public.

Attention has been directed to the vitamin B complex in foodstuffs, since the factors which make up this group are of great importance to India. The vitamin B_1 content of a number of foodstuffs has been determined biologically using the electrocardiographic method. Considerable work has been done in attempting to make Jansen's new chemical method of estimation applicable to foodstuffs, and when this method has been satisfactorily standardised it will be possible to determine the vitamin B_1 content of biological materials rapidly and easily. The method has been

applied to human urine with interesting results. An original method for determining nicotinic acid, which is known to be the pellagra-preventive factor, in biological materials has been devised and applied to a number of foodstuffs and also to urine. Preliminary results indicate that rice is richer in nicotinic acid than maize and this may explain why the rice-eaters rarely develop pellagra. It is not yet known whether a partial deficiency of nicotinic acid is an important defect of Indian diets.

The biological value of the proteins of some 30 common foodstuffs has been determined. The information now available on this subject has been summarised and published and may be regarded as sufficient for practical purposes.

Work has been carried out on the vitamin content of yeast grown on molasses. None of the specimens obtained so far have as high a vitamin B_1 content as brewer's yeast, and in the case of yeast used for medical purposes the concentration of vitamins is of great importance. Attempts have been made to increase potency by varying the conditions of culture, but so far without success.

Various animal experiments.

Further experiments on methods of improving Indian diets have been carried out, special attention being given to diets largely composed of tapioca, such as are consumed in certain parts of South India. It has been shown that deficiency of protein is the most important defect of such diets. This is not the case with diets of poor quality based on rice.

A monkey house has been built and experiments with monkeys begun. The effect on monkeys of long continued feeding on diets resembling those consumed by human beings in certain parts of India is being studied. Monkeys do well in Coonoor and the use of this animal opens out a whole new field for investigation.

Pathology.

The pathology of the skin and nervous system has been further investigated. The problem of the relation between dietary deficiency and pathological changes in the nervous system is of clinical interest. In this connection, the polarized light technique has been successfully used for the detection of Wallerian degeneration of the nervous system of deficiently fed animals. Fairly extensive myelin degeneration is constantly observed in the nervous system of animals fed on diets deficient in vitamin A; such degeneration usually occurs without the co-existence of nervous symptoms and may to some extent persist after long continued treatment with large amounts of carotene.

An investigation of changes in unaffected parts of the stomach and duodenum occurring in human cases of peptic ulcer has been carried out in collaboration with Dr. Ian M. Orr. Much of the material for examination was obtained during laparotomy for peptic ulcer. The most striking change observed was lymphocytic infiltration and hyperplasia of the lymphoid tissue in the mucosa. Particular attention was given to Auerbach's plexus in order to ascertain whether degenerative changes in the plexus precede lesions of the mucosa. Degeneration of the plexus has been constantly observed, but no light has been thrown on the significance of such degeneration in relation to lesions of the mucosa. Degenerative changes in the plexus have been noted in dogs and rats fed on diets containing a large proportion of tapioca; similar changes have, however, been seen in animals fed on other deficient diets. It has not proved possible to produce

peptic ulcer in animals by feeding them on diets based largely on tapioca.

Biochemical research.

The relation between vitamins and enzyme activity is a problem of physiological importance, because enzymes take part in the oxidation-reduction processes in the tissues. The enzyme phosphatase controls the deposit of calcium phosphate in bone and regulates other phases of calcium and phosphorus metabolism. It has been shown that phosphatase activity is inhibited by a combination of vitamin C and copper, while it is unaffected by vitamin C or copper alone. It appears that the enzyme is inhibited only when the vitamin is oxidised; there are, however, protective substances in the tissues which prevent oxidation. This finding is of importance because vitamin C and phosphatase occur together in the tissues.

Various other lines of biochemical lines have been followed.

Field work.

(a) *Diet surveys* have been carried out in Madras City, Delhi Province, the United Provinces, the Central Provinces, Bengal, Calcutta, Orissa, Assam and Kangra. Data about state of nutrition have been collected in the course of these surveys, the majority of which have been in rural areas. A survey of families with leprosy in Madras revealed that these families consumed an extremely deficient diet. A point of interest was the relative freedom from dental disease of children examined in Delhi Province. It will be advisable, in the near future, to publish the mass of information about food habits, etc. which has been collected, in concentrated and accessible form for the benefit of public health and agricultural departments, etc. These surveys are proving of great value in defining the problem of nutrition in India. The country is being mapped out from a dietary point of view and the defects of diet in different areas clearly demonstrated.

The examination of nearly 5,000 children has established a definite relation between certain signs of deficiency disease and selection by the A.C.H. index of nutrition. The index has been recommended as a useful supplementary method of assessing the incidence of malnutrition in a group of children between 6 and 12.

(b) *Controlled diet experiments.*—Previous experiments had shown that the nutritive value of the poor South Indian diet for rats could be improved by the addition of a calcium salt. It has now been observed that children living on diets largely composed of rice and consuming little or no milk can be benefitted by the regular administration of calcium lactate. Children receiving 0.5 to 1.0 gramme of calcium lactate for 3-5 months showed significantly greater increase in height and weight than children not receiving calcium. This result was confirmed in two different schools. In a nursery school the height and weight increments of groups receiving and not receiving the supplement were as follows :—

	Groups of children on an unsupplemented South Indian diet consisting largely of rice.	Control group on the same diet + calcium lactate.
Weight increase in 4-5 months.	0.98 lbs.	2.19 lbs.
Height increase in 4-5 months.	0.72 inches.	0.86 inches.

The differences in weight and height increases observed in this and other similar experiments were statistically significant.

(c) *The treatment of stomatitis.*—Stomatitis, resembling in certain respects the stomatitis of pellagra, is common in malnourished individuals in India. It is often associated with lesions of the muco-cutaneous junctions of the anus and vagina. This condition can be cured by substances rich in the vitamin B_2 complex such as yeast and milk. Evidence has accumulated in America that nicotinic acid produces a rapid cure in pellagra. Stomatitis does not, however, respond dramatically to nicotinic acid, and it therefore follows that it is not a pellagrous condition. Deficiency of another factor in the vitamin B_2 complex may be concerned.

(d) *The dark adaptation test for vitamin A deficiency.*—Considerable attention is being given to this method of detecting incipient malnutrition by workers in Europe and America. Conflicting reports of the value of the test are now appearing in the journals and fallacies are coming to light. It has been applied to groups of children in South India and the results obtained suggest a high incidence of vitamin A deficiency. At present, however, the test cannot be recommended for general application.

Basal metabolism.

It has been found that the basal metabolism rate of Indians living in Coonoor, 6,000 feet above sea level, is on the average 8 per cent. lower than that of Europeans living under similar conditions. A number of other investigators in India have also observed that the basal metabolism of Indians is 7.15 per cent. below European standards, and this point can now justifiably be regarded as settled. These observations are of importance in connection with the problem of calorie requirements.

The rice problem.

Study of the question in its various aspects was recommended by the League of Nations Inter-Governmental Conference on Rural Hygiene, 1937, and by the Nutrition Advisory Committee, I.R.F.A., in the report of its meeting in 1937. The present situation is that the use of highly milled rice is rapidly spreading all over the East ; the factors underlying the change have, however, never been fully analysed or understood, nor is there any clear idea as to its effect on the nutrition of the people. By means of questionnaires and field enquiries considerable information has been collected about the present situation in South India. The main conclusion seems to be that the habit of using home-pounded rice is rapidly disappearing or cannot be revived. The solution of the problem of ensuring that rice is consumed in such a form that the highest possible nutritive value is obtained must be sought along other lines.

Training course for health officers.

Early in 1938 a two months course in nutrition was given to 15 officers from various Indian States. Selected health officers from nearly all the principal provinces and States of India have now received training. Some of the officers are now actively employed in nutrition work.

Education and propaganda.

A few lectures have been given to suitable audiences. The nutrition film "Enough to Eat", was lent for exhibition in various centres. Press

notes on various aspects of nutrition were occasionally issued for the information of the public. Numerous enquiries continue to be received from departments and individuals; the labour involved in dealing with such enquiries is diminishing, both because of the long experience of the staff in preparing suitable replies and because publications are available to which enquirers can be referred. It is quite clear from the letters received in the laboratories, and from reports received by those in a position to know, that the educational work of the laboratories is meeting with success and that knowledge of the elementary principles of nutrition is rapidly spreading among the educated classes in India.

15. Additional nutritional enquiry by the Liaison Officer between human nutrition and agricultural research at the Imperial Agricultural Research Institute, New Delhi.

The entire programme of work for the liaison officer suggested by the third meeting of the Nutrition Advisory Committee of the Indian Research Fund Association, was given effect to during the year. The chief activities may be mentioned under the following heads :—

(1) Collection of evidence from medical authorities and public health workers regarding the state of nutrition of the villagers and townspeople.

Very little useful information could be gathered from different sources as it appears that no nutrition survey has yet been taken up in Sind, Orissa, Bombay, U. P., C. P. and North Western Frontier Province. Bihar has just started the work and an enquiry is in progress in the Kangra district of the Punjab. Bengal, however, has done some work in primary and secondary schools and also in some families. No information was available from Indian States.

(2) Survey of the quantities of food produced in various provinces in relation to the dietary requirements of the population.

Data regarding total yields of the principal food stuffs like rice, wheat, juar, barley, maize, bajra, ragi, gram, sugar, etc., in different provinces have been collected from various sources of information together with export and import figures. But it has not been possible to elicit any information regarding the total yield of different pulses, crop by crop, which form an important item in the dietary list all over India.

(3) The rice problem.

A preliminary investigation in the line suggested by the Nutrition Advisory Committee was taken up and a letter to that effect was addressed to the marketing survey office of all the provinces and of Burma, and some of the prominent States, to proprietors of some of the rice mills and also to the Secretary of the All India Village Industries Association. Personal enquiries were also made from manufacturers of rice mills during tours to the different provinces. A summary of the answers to the different questionnaires so far received from the different sources has been prepared.

(4). A review of the literature on the nutritive value of pulses.

This has been prepared.

(5). EXPERIMENTS ON THE NUTRITIVE VALUE OF PULSES AS SUPPLEMENT TO DIETS BASED LARGELY ON CEREALS.

(a) Two ounces of sprouted Bengal gram in addition to black gram (phaseolus mungo), dahl arhar (cajanus indicus) 2 ounces each, on a diet based on a mixture of whole wheat flour and raw polished rice (8 oz. each) together with some vegetables and spices, when administered to rats proportionately, enhanced the nutritive value of the above diet, as shown by increase of 17 g. weight over another batch without the supplement in 8 weeks.

(b) An experiment to compare the nutritive value of the five pulses commonly used in India, viz. lentil (lens esculenta), Bengal gram (cicer arietinum), green gram (phaseolus radiatus), black gram (phaseolus mungo) and red gram (canjanus indicus) together with rice alone in the ratio of 1 : 2 (the former in a sprouted condition to supply vitamins A and C) on batches of young rats weighing 33 g. on the average showed that the red gram group had an increase of 20 g., Bengal gram group 15 g. green gram and black gram 12 g. each and lentil only eight grams after four weeks. But unfortunately more than half of the experimental animals died without any apparent cause on the 5th week and this high death rate continued for the next week too. Postmortem was done on all the animals immediately after death and nothing abnormal was noticed except congestion of the various organs as liver, kidney, etc. Histological examination of the section of the glands as also those of the still surviving animals (that were killed under chloroform anaesthesia) revealed intense congestion of both liver and kidney, degeneration of the liver cells and distortion and disquamation of the epithelial cells of the renal tubules, which all show that some unknown toxin that was at work was responsible for the death of the animals from the fifth week onward. The same experiment was repeated with five batches of young rabbits but with no better success as almost all of them died from 12th to the 17th day of the experiment proving thereby a diet containing a big quantity of pulses for some time continuously was too toxic for the system to tolerate. So it is concluded that the proportion of pulses in the diet must not exceed some reasonable limit although diet surveys have shown that intake of pulses in various parts of the country might be increased, with resulting improvement in the nutritive value of diets based largely on cereals.

(6). EXPERIMENTS ON THE EFFECTS ON RATS OF SUPPLEMENTING THE POOR SOUTH INDIAN DIET WITH CALCIUM AND PHOSPHORUS.

Two experiments in this line show that the poor South Indian diet can be very effectively supplemented by addition of small quantities of calcium lactate, or by a combination of calcium lactate and alkaline potassium phosphate or preferably by a single salt like basic calcium phosphate. Addition of alkaline potassium phosphate alone had no supplementary effect.

(7). TOURS.

The agricultural colleges, research institutes and prominent experimental farms of Assam, Bengal, Bihar, C. P., U. P. and Punjab were visited in order to gain first hand information regarding the economics of agricultural and nutritional problems. Different parts of the Tehri Garhwal were also visited to study the relation of dietary deficiency with the high prevalence of leprosy in that locality.

16. Enquiry on cheap well balanced diets under the Bombay Presidency Baby & Health Week Association.

The enquiry is intended to conduct further investigations on the balanced diets advocated in pamphlet No. 8 of the Bombay Presidency Baby & Health Week Association with a view to improving upon them without materially increasing the cost. The investigation is being pursued simultaneously along two lines (i) nutritional and (ii) analytical and experimental.

I. Nutritional.

2. Experiments with the balanced diet No. II, the physiologically ideal diet and reformatory (control) diet came to an end in November 1937. It was decided to continue our observations on the balanced and the reformatory diet for a further period of one year and to discontinue the ideal diet as the growth obtained with it was practically the same as with the balanced diet No. II. The group of boys on ideal diet were given the reformatory diet in 1938 in order to observe the effect of the return to this diet on the growth of the boys.

3. Laboratory experiments on rats had shown that fresh whole milk gave the best results and cannot be substituted by skim milk powder, red palm oil, butter, soyabeans etc. Fresh whole milk is also cheap in places outside Bombay.

4. Accordingly balanced diet No. III was formulated in which 9 ozs. of fresh milk replaced the skim milk powder and the butter. The soyabeans were replaced by dal (Gajanus indicus). The total proteins and fats of this diet are 106 and 84 gms., respectively, and animal proteins and fats in them are 28.2 and 30.5% respectively. The total calories are 3140. The cost of the diet at current prices is Rs. 7/- only, i.e., annas seven less than the former balanced diet No. II.

5. All the rations were correctly weighed out, cooked and served out equally to all boys under strict supervision. In addition to the usual individual record of height, weight, physical examination etc., a graphic record of the fortnightly progress of each boy was kept. The daily quantity of milk was received in the presence of the medical officer in the morning and distributed to the scheduled boys immediately in measured quantities. Butter was also similarly issued at the morning meal. The wastage in the plates, after meals, was weighed every day and recorded.

6. The balanced diet No. III and the "Control diet" along with the continuation of the last year's diets mentioned in para 2 have been the subject of our observation during the year 1938. The balanced diet No. III has been tested on fresh group of boys. The response in growth on balanced diet No. III is found to be in no way inferior to that obtained for balanced diet No. II in spite of the reduced cost.

II. Analytical and experimental.

7. During the course of the year, growth tests on albino rats were carried out with various food stuffs and in different combinations. The food stuffs tried out were fresh whole milk, skim milk powder, red palm oil, soyabeans, butter and vitamin D, singly and in combinations. The balanced diet was taken as the basal diet.

8. The conclusion arrived at was that fresh whole milk gave the best results and could not be substituted by any of the above articles of diet with advantage.

17. Enquiry into the basal metabolism of adults and children in Bombay by Professor S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

The basal metabolism of 24 men and 52 women in Bombay has been fully investigated with the help of the Sanborn basal metabolism tester. As mentioned in the last report each subject was tested on three days, three or four observations being made on each day. The results thus obtained are tabulated in three series. The average oxygen consumption in the first series was definitely higher than the average of the latter two series. The following tables show the results of basal metabolism experiments as well as certain other physical and physiological observations.

Table I Averages.

	Oxygen	Heat production		Deviation from standards in %		
	Consumption per minute c. c.	Total cals.	Per sq. m. per hour cals.	Mayo Clinic.	Harris Benedict.	Aub & DuBois.
Men series I	193	1342	33.8	—13.0	—8.3	—9.4
,, ,, II	186	1288	34.3	—16.4	—11.9	—13.3
,, ,, III	188	1305	34.7	—15.5	—11.5	—12.5
Women series I	156	1087	33.0	—8.5	—13.4	—11.4
,, ,, II	152	1060	32.1	—10.9	—15.8	—13.5
,, ,, III	152	1055	32.0	—11.2	—15.9	—14.1

Table II Averages.

Age.	24 Men. 22 years 8 months	54 Women. 22 years 0 months
Weight without clothes in Kg.	52.5	44.7
Standing height in cms.	166.0	152.7
Sitting height in cms.	86.2	79.6
Pirquet index or Pelidisi.	93.4	92.3
Respiration rate per minute during experiments.	17	17
Pulse rate per minute.	65	70
Blood pressure { systolic.	104	102
Blood pressure { diastolic.	63	64
Vital capacity in c. cms.	2552	1622

On the basis of these investigations the daily caloric requirements for men and women have been calculated; they amount to about 2600 and 1900 calories respectively.

In addition to the number of subjects mentioned above several others have been partially investigated. The work is in progress.

18. Ascorbic acid enquiry by Prof. B. C. Guha, at the University College of Science & Technology, Calcutta.

(1) Vitamin C and toxins.

The work on the effect of ascorbic acid on toxins *in vitro* and *in vivo* has been continued. The effect of graded quantities of ascorbic acid and other natural reducing agents like glutathione and cysteine on tetanus and diphtheria toxins *in vitro* has been more fully investigated and inactivation has been observed in all cases. In order to test, however, whether the detoxication observed is due solely to the lowering of pH of the toxins by the addition of ascorbic acid, a series of controlled experiments was carried out and it was observed that ascorbic acid has a specific effect on the toxins apart from the effect of lowering of pH, and among the different reducing agents ascorbic acid appeared to be the most efficient inactivating agent (1). In *in vivo* experiments, injection of ascorbic acid immediately before and after toxin injection gave favourable results. But the delayed administration of massive doses of the vitamin failed to protect the animals. The effect of oral administration as well as subcutaneous injection of vitamin C over a week previous to toxin injection indicates that the vitamin perhaps helps in increasing resistance in an indirect manner, namely by supplying vitamin C to replenish that which has been found by other workers to be destroyed in acute infections

(2) Vitamin C and bacteria and fungi.

Work on the effect of minute amounts of ascorbic acid on the growth of certain bacteria and fungi in synthetic media has been continued. Although glutathione and cysteine have also been found to inhibit the growth of the bacteria studied, namely, *B. coli*, *B. subtilis*, *Aerobacter aerogenes* and *Staph. albus*, the effect of ascorbic acid is by far the strongest.

In the case of certain fungi namely, *Aspergillus niger*, *A. oryzae*, *Sacch. cerevisiae* and *S. ellipsoidens* it has been found that the rate of growth during the first 3 or 4 days is slower in the absence of the vitamin than in its presence, but after the 5th day, the growth becomes approximately the same. It appears from parallel estimations of the vitamin C content of these fungi that they can themselves synthesise vitamin C, which stimulates their growth, but the culture to which the vitamin is added at the beginning of incubation naturally gets an advantage over the controls.

The effect of the inoculation of certain living pathogenic bacteria into susceptible animals, injected daily with vitamin C before and after bacterial infection is at present under investigation.

(3) The assessment of the level of nutrition with reference to vitamin C.

The saturation test of Harris and Ray for the assessment of vitamin C subnutrition has been fairly widely employed. Another method recently introduced is that of the intra-dermal skin test with 2 : 6 dichlorophenol indophenol. An investigation carried out to see how far the two techniques agreed showed a substantial measure of agreement. In order to test the accuracy of the skin test, an investigation was carried out with guinea-pigs and it was observed that there was gradual increase in the time of decolorisation in the skin test with the duration of feeding on a scorbutic diet; the time of decolorisation gradually diminished as vitamin C was

introduced into the diet. The skin test is very much simpler than the method involving the estimation of urinary vitamin C, but its limiting factor is the degree of natural skin pigmentation.

(4) ASCORBIGEN OR COMBINED ASCORBIC ACID IN PLANT AND ANIMAL TISSUES.

As reported earlier a part of ascorbic acid in several plant foodstuffs has been found to be present in a combined form, which has been called "ascorbigen". The anti-scorbutic potency of the substance has now been proved by experiments with guinea-pigs and it has been extracted from dried cabbage by chloroform. Its existence in the tissues like liver, kidney, brain and small intestine of normally fed guinea-pigs has been observed, and among these brain seems to have the largest content of ascorbigen. It has been possible to concentrate ascorbigen from cabbage by fractionation and absorption methods. Further studies on its concentration with a view to isolation and also on its distribution in different plant and animal tissues are in progress. Ascorbigen has also been found in urine and it seems that all estimations of urinary vitamin C should take this into account.

(5) ESTIMATION OF VITAMIN C.

A method of estimating "total" vitamin C comprising free ascorbic acid, dehydro-ascorbic acid and ascorbigen with the elimination of interfering substances has been developed, and it would appear in the light of new facts that the original method of simple titration with the indophenol indicator would not give sufficiently accurate results. Ascorbic acid oxidase, which has been used as an analytical reagent in this estimation has been prepared either from cucumber or from white gourd. An investigation of the total vitamin C in a considerable number of foodstuffs by the new method has been carried out.

(6) VITAMIN C AND LUTEINISING AND FOLLICULAR HORMONES.

It had been claimed by Bourne that the injection of the luteinising hormone into female guinea-pigs fed on a scorbutic diet prevented the onset of scurvy, suggesting a relation between vitamin C and the luteinising hormone. The corpus luteum is, of course, known to contain a large amount of vitamin C and it had also been claimed that scurvy is delayed in pregnant guinea-pigs. A detailed investigation on this subject has, however, revealed no effect of pregnancy and of luteinising hormone on the onset of scurvy in guinea-pigs. It has also been observed that the injection of luteinising and follicular hormones into normal female guinea-pigs does not appreciably alter the vitamin C content of the tissues like liver, spleen, kidney and small intestine.

19. Studies in human metabolism by Dr. K. P. Basu, Dacca University.

This enquiry is concerned with the physiology of human nutrition, specially with the metabolism of proteins, calcium and phosphorus in human subjects with a view to getting better knowledge about the requirements of these essential elements by Indians and finding how these requirements are met by typical Indian dietaries. Chemical analysis and animal experiments, no doubt, give some idea regarding the nutritive value of foodstuffs but the quantitative requirements of the different elements and the actual utilisation of food materials can only be decided by human

experiments. The Europëan standards will not probably hold good for Indians.

Protein.

(*a*) *Nitrogen elimination on protein-free diet* :—An experiment was arranged to study the minimum nitrogen expenditure of an individual who was kept on a protein-free diet containing adequate energy supplying materials. The total urinary nitrogen excretion of one of the experimental subjects (G.C.N., 49 kilo) was 1.499 gm. or 0.0306 gm. nitrogen per kilogram body-weight. The values obtained by Martin & Robinson were 0.038 gm. (C.J.M.) and 0.35 gm. (R.R.) The faecal output in the present case was 0.946 gm., while Smith found it to be 0.91 gm.

(*b*) *Protein requirements for balance* :—A large number of experiments were performed with diets containing varying amounts of protein derived either from rice or from whole wheat (atta) and with diets so chosen that the dietary nitrogen was equal to the total nitrogen excreted in urine and faeces. On rice diet G.C.N. (aged about 20 years) required about 28 gm. of protein i.e. 40 gm. protein per 70 kilo body-weight for nitrogen equilibrium, while S.N.D. (aged about 24 years and body-weight 46 kilo) required about 37 gm. protein or 56 gm. protein per 70 kilo body-weight. On atta diet, however, S.N.D. required 44 gm. of protein or 67 gm. of protein per 70 kilo body-weight for nitrogen balance. It might be pointed out here, that S.N.D. required more protein than G.C.N. for balance. Hence in order to get a more representative value for protein requirement for maintenance of Indians, a large number of experiments on different individuals must be carried out.

Now the question arises if the typical dietaries of the poor vegetarians satisfy their protein requirements. Experiments have been performed to settle this point. It was found that G.C.N. maintained himself in nitrogen balance on diets (i) containing only 250 gm. rice and 25 gm. pulse and (ii) on a diet containing only 150 gm. atta and 25 gm. pulse. (The diets always contained 200 gm. vegetables, 100 gm. sugar, 25 gm. fat and sufficient energy giving material in the form of sago). On the other hand, on typical diets generally used by people and containing 600 gm. of either rice or atta, he daily retained about 1.6 gm. nitrogen or 10 gm. protein on rice diet and about 5 gm. nitrogen or 31 gm. protein on whole wheat diet.

Similarly, S.N.D. could just maintain his nitrogen equilibrium on diets containing 400 gm. rice or 300 gm. atta, while on 500 gm. rice or atta diets he retained considerable amount of protein.

(*c*) *Biological value of proteins of rice and wheat diets at different levels* :—The diets always contained a definite amount (25 gm.) of pulse, and 200 gm. of vegetables. The cereals were fed at different levels, energy intake being kept constant by the incorporation of sago, which is nitrogen-free. At an intake of 250 gm. of rice, the mean biological value of the proteins was 81, at 400 gm. intake, B.V. was 74 and at 600 gm., B.V. was 68. In case of wheat diets, the mean biological values were 75, 72 and 67 at 150, 400 and 600 gm. of atta intake respectively.

Calcium and phosphorus.

Balance experiments of calcium and phosphorus with rice and wheat were conducted on three healthy Bengali youths. B.C.R.S. (a university student of 54 kilogram body-weight) showed a urinary excretion of 70 mg. calcium and a faecal elimination of 162 mg. calcium on

a typical Bengali rice diet. The intake of calcium was 280 mg. and the experimental subject showed a retention of 48 mg. When this diet was supplemented with 1 lb. milk, containing (570 mg. calcium) urinary excretion rose to 160 mg. faecal excretion to 566 mg. with a retention of 174 mg. showing the remarkably beneficial effect of the addition of milk to such a diet. The requirement of calcium per 70 kilo body-weight was calculated to be 0.29 gm.

G.C.N. on a diet of 600 gm. rice with a calcium content of 152 mg. excreted 25 mg. of calcium in urine and 160 mg. in faeces and consequently was on a deficit calcium balance of 33 mgm., whereas on a similar diet of 600 gm. atta (containing 310 mgm. of calcium), he excreted 185 mgm. of calcium in urine and faeces and hence retained 125 mgm. This shows the superiority of atta over rice diets so far as calcium metabolism is concerned. The substitution of a part of rice of the rice-eaters' diet by atta should, therefore, be recommended.

S.N.D. showed total excretions of calcium varying from 315 to 451 mgm. and the calcium requirement per 70 kilogram body-weight was calculated to be 0.58 gm. Both with rice and atta diets calcium intake fell short of S.N.D.'s requirements and the subject was in negative balance by about 200 mg. on rice diet and about 140 mgm. on atta diet. This shows that some calcium rice foodstuffs like milk should always be incorporated in diets.

Requirement of phosphorus for G.C.N. was found to be 0.638 gm. which came out to be 0.91 gm. per 70 kilogram body-weight, while the corresponding value on S.N.D. was 0.764 gm. which when converted to 70 kilo body-weight, was calculated to be 1.16 gm. It was interesting to find that on both rice and wheat diets, the experimental subjects were always on positive phosphorus balance. B.C.R.S. on a typical Bengali diet showed an average elimination of 1.093 gm. phosphorus and a retention of .304 gm.

Uric and oxalic acids.

Estimations of daily eliminations of uric and oxalic acids in urine on various diets were continued this year also. Elimination of uric acid was found to be roughly constant at about 515 mgm., while the elimination of oxalic acid showed no regularity on different diets. Data of the eliminations of calcium, phosphorus, uric and oxalic acids in urine on rice and atta diets do not seem to reveal anything as to their importance as probable factors in the formation of urinary calculi.

Creatinine.

Creatinine was daily estimated and found to be uniformly constant at 1.02 gm.

20. Peptic ulcer enquiry by Dr. Ian M. Orr.

I. During the year the experiments commenced in 1937 matured and the work of the year consisted in assessing the significance of the findings and relating them to peptic ulcer pathology as seen in western countries. One paper in conjunction with Dr. Rao of the Nutritional Research Institute, Coonoor, has been submitted to the Indian Journal of Medical Research. This paper sets out in detail the experimental methods employed and the results obtained in the study of the pre-ulcerous pathology of peptic ulcer in South India.

By the courtesy of the Professor of Pathology, British Post-Graduate Medical School, London, accommodation was provided and facilities given for the study of stained sections and the preparation of microphotographs.

Six sets of sections obtained and prepared in India were examined as follows.

(a) Thirty-nine sections taken from stomachs in which there was peptic ulcer as found in South India.

(b) Ten sections removed from stomachs, not the seat of ulcer, but where the patient had suffered from ulcer symptoms in South India.

(c) Nine sections representing the normal Travancore stomach.

(d) Seven sections representing the normal North Indian stomach.

(e) Twenty sections of stomachs and duodenums from rats in the Coonoor Institute after feeding for varying periods on the South Indian and Travancore diets.

(f) Twenty four sections taken from dogs' stomachs before and after putting them into a strict tapioca diet.

In the thirty-nine specimens examined only four showed normal mucosa. The remainder showed some degree of infiltration with plasma cells and lymphocytes. In the more advanced degrees of invasion the mucosal glands were separated and reduced in number, the intervening spaces being crammed with lymphocytes. Aggregations of lymphoid elements occurred to form follicles and in some sections the whole of the submucosa was one lymphoid area, with aggregation into follicles at intervals.

The follicles frequently showed abscess formation with spaces in the centre filled with necrotic cells and in some sections follicles were seen to have ruptured into the surface forming superficial ulcers. Round celled infiltration of the muscle layers was present to some extent in certain sections.

The ganglia of Auerbachs plexus were much more prominent than normal and the ganglion cells were swollen and the cell outline indistinct. The nucleus had often disappeared or appeared only as fragments of chromatin. When complete nuclei were found they were swollen with fragmentation of the nucleolus. Occasionally lacunae empty of cells were found in the degenerating ganglia. Ganglia varied in the degree of change, some being completely degenerate while in others the changes were slight, though in no case was a normal plexus observed.

In all cases there was considerable infiltration of the plexus with round cells and the glial cells of the plexus were increased in number. In the more advanced conditions the entire plexus was surrounded by a thick capsule.

II. It has been noted that frequently a clinical condition simulating ulcer occurs but in which no ulcer can be demonstrated by X-ray or at operation. These cases are believed to be pre-ulcerous conditions and in order to investigate the pathology of this condition, cases showing such symptoms were operated upon and portions removed from the stomach and duodenum for histological examination. In ten such studies it was found that mucosa was infiltrated with lymphocytes. In six lymphoid follicles had formed and some showed evidence of bursting into the surface.

Changes in Auerbachs plexus identical with those described in the previous section were noted in all.

III. In order to ascertain if the conditions referred to in the previous sections were common to the population of Travancore (where ulcer of the duodenum is particularly common) sections were obtained post mortem from cases which died by violence, that is cases in which there was no reason to suspect gastric disease.

Eight specimens were examined, several sections being obtained from each specimen. As in the previous groups all the sections showed lymphocytic invasion of the mucosa and one showed lymphoid follicles distended and hypertrophied similar to that found in definite ulcer cases. Six specimens could not be examined with a view to demonstrate Auerbachs plexus as the fixation was done some time after death and post-mortem changes had taken place in the ganglion cells, but two cases were the result of death under an anaesthetic and fixation was carried out early, and in both, early changes in Auerbachs plexus were noted. These changes were characterised by fragmentation of the nucleolus in some of the ganglion cells, slight invasion of the plexus by fibrous tissue cells and lymphocytes and plasma cells.

The inference drawn from these studies is that the stomachs and duodenums of the people in Travancore, where peptic ulcer is a common disease, have chronic changes in the mucosa and the intra-mural nerve plexuses which may account for the hypermotility and pylorospasm so common among them and reported in this enquiry in 1937.

IV. Control specimens were collected from stomachs in North India. These showed healthy formation of the mucosa and a complete absence of ganglion cell degeneration and plexus invasion.

V. An attempt was made to reproduce in rats the changes noted in South Indian stomachs by feeding them on the South Indian diet. Sir Robt. McCarrison in 1931 produced peptic ulcer in albino rats by feeding them on the diet of Travancore and Madras. The experiment was repeated with the same strain of rats in the Nutritional Research Institute in Coonoor but instead of waiting till the rats died before exploring their stomachs, they were killed off by injecting air into the heart after a period of months on the diet, the object being to ascertain the earliest recognisable change in the rat's stomach before ulcer developed. A control group fed on the stock diet were examined at the same time.

The first change observed in a rat after one month on the Travancore diet was a tendency to degenerative changes in the ganglion cells of Auerbachs plexus. While the majority of the cells were normal, about one third of them showed fragmentation of the nucleolus, pyknosis and other early signs of degeneration.

In a rat killed after three months, half the cells showed early degeneration and a small number were completely degenerated with absence of the nucleus. As the length of time on the diet increased the degenerative changes became more marked and in the rats fed for six months on the Travancore diet, infiltration of the mucosa was observed and this infiltration became more marked in later specimens and extended to the plexus itself. Rats were killed at one, three, six, eight, fourteen and sixteen months and the progress of disease noted.

The conclusion drawn from this series of experiments is that the Travancore and Madras diets do cause changes in the stomach and duodenum leading up to ulcer and that the earliest change is a degeneration of Auerbachs plexus and that round celled infiltration follows the degeneration and does not cause it.

VI. The last experiment was with dogs. Seven dogs were subjected to a laparotomy and a small portion of the stomach and duodenum excised to form a standard of normality . The dogs were then put into a diet consisting mainly of Travancore tapioca and at the end of two months those which survived were again operated upon and portions removed for examination. This was repeated at four months and then the experiment was abandoned as the surviving animals were in a very poor state of health.

In the healthy animals the plexus of Auerbach was seen as a group of large ganglion cells lying between the muscle coats. The ganglion cells were accompanied by a few glial cells and there was little or no infiltration with round cells, plasma cells, or fibroblasts. The ganglion cells were pear shaped with one and occupied by the nucleus. The nucleus was rounded clear and refractile with a well marked nucleolus and a well marked nuclear membrane. Two months after the diet the cells became pyknotic or degenerated and there was marked invasion with round cells and a capsule formed round the ganglion rendering it much more obvious. The condition became comparable to that seen in the human ulcer sections and pre-ulcer sections described in (a) & (b.)

It is interesting to note that the cause of death in two cases was intusscusception and in two cases ascites developed.

Final conclusions of the peptic ulcer enquiry.

The effect of the year's work has been to find in histopathology an explanation for the clinical phenomena described in the report of the Peptic Ulcer Enquiry in 1936-1937. These clinical phenomena may be summarised as follows :—

(1). Peptic ulcer is sixty times more common in South India than in North India.

(2). The highest incidence in South India is in the centre and north of Travancore.

(3). The diet of the district is markedly deficient in protein and vitamins A & B.

(4). Test-meal examinations carried out on normals in South India, the Deccan and in North India showed that the South Indian stomach is commonly affected by chronic gastritis, and duodenitis and possibly for this reason the acidity though high is not excessive.

(5). X ray examinations of normals in South India, the Deccan and in North India show that in South India a high percentage of stomachs are spastic with pylorospasm and hyperperistalsis.

(6). A large proportion of those who complain of dyspeptic symptoms have no actual ulcer but a condition of irritable duodenum and antral gastritis which may go on to ulcer formation later on.

The histopathology described in this report suggests that the process is as follows :—

(1). Deficiency of protein and vitamin A & B in the diet of South India affects the nutrition of the intra-mural ganglia which control the movements of the stomach and the secretions and maintain the balance between sympathetic and vagal influence. The failure of the myenteric plexus to perform its normal function leads to a condition of vagal preponderance, resulting in pylorospasm hyperperistalsis and hypersecretion.

At the same time the dietetic deficiency, probably due to the lack of vitamin A, lowers the resistance of the gastric mucosa to infection and this leads to lymphocytic infiltration and follicular hypertrophy. In the more advanced cases the follicles become abscesses which rupture onto the mucosal surface forming microscopic ulcers. The hypersecretion hyperperistalsis and pylorospasm contribute to the prevention of these ulcers healing, and the spastic contraction of the pylorus on these mucosal erosions leads to the dyspepsia typical of the pre-ulcer stage. Finally the microscopic ulcers coalesce to form a large chronic ulcer.

This enquiry has not established any valid explanation as to why the ratio of ulcer in South India is ninety-five men to five women. Certain suggestions have been made that women have more frequent meals, being in the house all day, and that in the poorer homes the women are reduced to eating the millings or bran of the rice and drinking the rice water, thereby deriving certain sources of nourishment denied the men. There is probably a deeper biological explanation than this connected with the influence of the sex glands on the tone of the stomach. (It was noted in a small series of barium meal examinations on normal Travancorean women that the stomach lacked tone and motility when compared to the male stomach). It has been noted by Matzner that injections of Antuitrin S have a marked protective effect in resisting attempts to produce experimental ulcer and in speeding up the healing of ulcer.

The final conclusion of the peptic ulcer enquiry is that the lack of protein and vitamin A & B in the South Indian diet is the direct cause of the high incidence of peptic ulcer and that the addition of milk and milk products, cereal grains, and green vegetables to the South Indian diet would protect the gastro-duodenal mucosa from the nerve degeneration and inflammatory change which precede ulcer.

LINE OF STUDY SUGGESTED FOR A FUTURE ENQUIRY.

(a). Follow up of a series of children at present receiving the benefit of a diet supplemented by milk either whole or dried, to ascertain whether or not they remain free from pre-ulcer symptoms and signs in adult life. X ray and test meal investigations might be made on such children after four years on the supplemented diet and comparisons made with the children who have been brought up on the usual diet of South India.

(b). A detailed investigation to find out why women are affected by peptic ulcer comparatively rarely.

21. Researches on the working efficiency of Indian labourers under Professor W. Burridge, Professor of Physiology, King George Medical College, Lucknow.

The experiments have been performed with a Martin's bicycle ergometer. The subject's resting respiration is first determined while he is seated on the saddle by collecting his respired air in a Douglas bag. The volume of this air is measured and its composition determined by a Haldane gas analysis apparatus.

Next the subject pedals at the wheel of the cycle in time with a metronome for a given period and his expired air again collected. The bicycle ergometer gives us the measure of the work done, and the differences between

the resting and working expirations give its cost to the individual concerned. From these figures the subject's mechanical efficiency is determined.

The research-worker is a healthy young man of 25 and good zamindari stock. The first group of experiments, and they were many, was therefore done on him, and showed that mechanical efficiency gradually rose with the speed of cycling up to a certain maximum, and that after that efficiency rapidly fell. The respiratory quotient was found to rise with the speed and to be above unity when efficiency fell. The work done on laboratory workers indicates that each individual has his own speed of maximum efficiency. The work done on labourers so far shows a lower speed of maximum efficiency and a lower maximum than the laboratory workers. But as all the laboratory workers were cyclists, and all the labourers were not we require much more evidence before drawing any sort of conclusion.

22. Enquiry into malnutrition amongst Bengali boys with regard to vitamins C and B, by Professor N. M. Basu at the Physiological Laboratory, Presidency College, Calcutta, in the year 1938.

Vitamin C enquiry.

The following investigations were carried out :—

(*a*) *The normal urinary output of vitamin C in 24 hours* :—The normal urinary output in 24 hours of free, dehydro and combined ascorbic acid of 17 boys were examined. Excepting 4 boys who were taking plenty of fruits or vegetables or both, all the others had an average daily output of less than 15 mgms. of free ascorbic acid, (but a majority of these had an output of less than 10 mgms. per day) varying between 1.5 mg. to 13 mg. Harris and Ray's figures for daily excretion range from 15 to 25 mgms. depending on the adequacy of the diet.

The excretion of dehydro and combined ascorbic acid is more or less erratic, apparently not depending on the diet, for it was noticed that neither de-hydro nor combined ascorbic acid was present in the urine of one subject even after the continued ingestion of large amounts of vitamin C and an extract of various vegetables, such as beans, tomatoes, cabbages etc. which are known to be rich in combined ascorbic acid.

(*b*) *The state of saturation of Bengali boys, both Hindu and Mahomedan, with regard to vitamin C* :—The extent of deficiency or the condition of saturation of boys was determined by the total amount of ascorbic acid required to be ingested before their urinary excretion reached a constant figure. In those cases which exhibited a very low state of saturation, the administration of this vitamin was continued till there was 30% excretion of the amount ingested on the previous day. The vitamin was administered either in the form of fruit juices, supplemented by vitamin C crystals when necessary, or in the form of standardized tablets of vitamin C.

It was found (1) that 2 boys whose normal urinary output was above 40 mgms. were in a state of saturation (2) that the others, whose normal urinary output varied between 2.4 and 28.0 mgms. were in different states of saturation, viz., (a) those excreting normally over 15 mgms. but below 30 mgms. daily requiring less than 500 mgms. for attaining the state of saturation in which the amount excreted was 60% of the amount of vitamin C ingested, (b) those excreting normally per day between 10 to 15 mgms.

requiring nearly between 1000 to 1500 mgms. for attaining the same state of saturation and (c) those excreting normally per day below 10 mgms. requiring between 1500 to 3000 mgms. for attaining the lower state of saturation.*

(*c*) *Course of excretion of this vitamin after its administration in large doses for several days and the time taken for its output in urine becoming normal* :— It was found (1) that after the administration of a large dose of vitamin C the percentage of excess excretion in the first period of 12 hours depended on the state of saturation of the subject, for it was generally found to be nearly 60% towards the beginning of the experiment, and 70% towards the end of the experiment when the subject nearly reached the state of saturation, but never 85% as mentioned by Hawley and Stephens, and (2) that the initial rate of excretion was reached within about 24 hours, as mentioned by Johnson and Zilva, only in cases of persons who were not in the habit of taking fruits.

(*d*) *Minimum requirements of this vitamin* :—These were determined in the following way :—The boys were brought to a state of saturation by the ingestion of large doses of ascorbic acid, and then the intake of this vitamin was continually reduced till a minimum dose was found at which the rate of urinary excretion was maintained at nearly 30% of the intake for several days.

The results of investigation on six boys showed that they required on an average 50 mgms. per day.

(*e*) *Relationship between the Cu-content and the ascorbic acid oxidase activity of the different protein fractions separated from the drumstick juice* :— Stotz et al asserted that the activity of the ascorbic acid oxidase in oxidising vitamin C is mainly, if not solely, due to its Cu content and is proportional to it. This question was studied incidentally in the drumstick juice which was shown by Srinivasan to be rich in ascorbic acid oxidase. As there are various proteins in this juice and as enzymes generally remain adsorbed with protein, it was necessary to ascertain (1) with which protein fraction this hexoxidase remains principally adsorbed, and (2) what relationship exists between the Cu content of this fraction and its hexoxidase activity. This investigation was carried out in the following way :— All the proteins of the drumstick juice were precipitated by saturation with $(NH_4)_2SO_4$. This precipitate was then treated successively with Cu-free distilled water, 5% NaCl solution, 0.4% NaOH solution and 75% alcohol, Cu-free distilled water being used in the preparation of these solutions. The filtrate obtained after each treatment was examined for its hexoxidase activity and Cu content. It was found that although there was general correspondence between the hexoxidase activity and the Cu-content of each fraction, the proportional relationship could not be confirmed.

On account of the scarcity of drumstick during rains and the subsequent months these experiments could not be repeated.

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* According to J. B. Youmann, after the administration of a large dose, say 600 mgms. if there is 30% excretion the person is said to be in the lower limit of state of saturation under ordinary conditions. The amount required for reaching the state of saturation is obtained by adding up the amounts of Vitamin C given day by day till the saturated condition is attained and deducting therefrom the excess excretion of Vitamin C during the period of administration.

VITAMIN B_1 ENQUIRY.

This work has been already taken up and the assay of vitamin B_1 content of urine of boys is being investigated by the rat-growth method.

23. Researches on calcium deficiencies by Dr. D. C. Wilson.

The following investigations were carried out during the year in the Kangra Valley. (Punjab).

In the field work I have had assistance from the following workers of the I.R.F.A. :—

Dr. Narinder Singh	(diet surveys).
Dr. K. L. Shourie	(blood examinations).

1. AT LAUNA VILLAGE.

(a) Diet Surveys of 38 families, of which 23 contained at least one member suffering from one of the forms of rickets (early rickets, late rickets, or adult rickets (osteomalacia). In addition 12 individual diet records were obtained. Information was obtained also about seasonal variations in diet.

(b) Nutritional examination. A thorough study was made of all members of the 39 Launa families, and

(c) Haemoglobin estimations carried out on these.

(d) Specimens of food-stuffs from the Launa area have been dried and sent to Coonoor for analysis.

(e) General observations were made regarding seasonal variations in the incidence of deficiency conditions in the Kangra Valley.

(f) Launa water has been examined for calcium and phosphorus.

(g) Blood examinations have been carried out of 37 members of Launa families for serum calcium, inorganic phosphorus and phosphate.

(h) Vitamin D_2 (irradiated ergostert or calcifert) and Vitamin D_3 (irradiated 7-dihydro-cholesterol) have been tested out clinically for comparison of 32 cases of osteomalacia, on behalf of the Therapeutics Trial Committee of the Medical Research Council.

2. IN HAMLETS NEAR THARL, PALAMPUR DISTRICT IN THE MAIZE GROWING AREA.

A diet survey was carried out on 20 families, and every member examined of signs for deficiency disease. Eleven families had one or more members suffering from osteomalacia, one case of pellagra was found.

The results of the nutritional examinations and the records of the diet surveys have all been sent to Cambridge, where they are now being worked out by my clerk (Miss Ellis B.Sc.) under the direction of two workers under the Medical Research Council, Dr. McCance and Miss Widdowson in the Department of Medicine.

24. Bengal fish enquiry by Prof. B. C. Guha at the University College of Science and Technology, Calcutta.

A nutritional investigation of Bengal fish has been carried out with reference to protein, fat, ionisable iron content and total mineral content of 30 different varieties of fish. It has been found that the water content of the fish varies from species to species from 40 to 80%, protein content from 13 to 22%, body fat from 2 to 5%. The estimation of iron, calcium, phosphorus and copper in the ash has been carried out along with the ionisable iron content of the raw fish.

25. Enquiry into calcium metabolism in health and disease by Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

A study of the literature revealed contradictory views concerning the influence of vitamin D on the absorption of calcium from the intestines and its excretion into it. It was therefore decided to investigate the absorption of calcium from the intestines of albino rats, in first instance, and later to extend the observations to other animals. The following technique was adopted.

Rats chosen for experiment were deprived of food for 16-20 hrs. before the experiment. They were then subjected to ether anaesthesia and the abdomen opened. Cannulae were introduced near the pyloric end of the duodenum and at the ileo-caecal junction. The contents were washed out by warm saline till the fluid was free from calcium. A known amount of solution of calcium salt was introduced by means of a syringe, the loop tied, the intestines replaced and the abdomen was closed. The animal was allowed to recover. After three hours it was again anaesthetised, the loop removed and its length measured. The lumen of the loop was then washed with large amounts of saline, and the amount of calcium in the washings estimated.

The same technique with minor modifications was followed when investigating the excretion of calcium in the small intestines, caecum and colon. Several such experiments were carried out on normal rats, rachitic rats, rats protected from rickets by adequate dosage with irradiated ergosterol and rats suffering from the administration of toxic doses of irradiated ergosterol.

The number of experiments performed to date though fairly large is considered not enough to permit drawing of definite conclusions.

In addition to these experiments the calcium and phosphorus content of tissues of normal albino rats is being compared with that of rachitic rats.

LEPROSY

26. Leprosy enquiry by Dr. J. Lowe at the School of Tropical Medicine, Calcutta.

(a) CLINICAL WORK.

i. The study of the different forms of leprosy and their relation to

each other has been continued, the findings being checked by histological studies. This study has been discussed in previous reports.

ii. A brief study of leprosy in Burmans compared with leprosy in Indians. The chief findings are as follows :—In Burmans the severer lepromatous leprosy forms about 50% of the total compared with about 25% in Indians. In Burmans the mild neural cases appear to show a more marked tendency to develop into the severer forms. In Burmans the mild forms of the disease are found chiefly in children whereas in Indians they are found in all age groups. The conclusions are that leprosy in Burmans is more often progressive, serious and infectious. This is attributed to low racial immunity.

iii. A study of seasonal variations of leprosy in Calcutta. The study is made on the basis of clinical and bacteriological examination of about 3,000 cases of leprosy seen at different seasons of the year during the last two years. The seasonal variations are seen markedly in lesions of neuro-macular type. In such lesions, during the months of February to September, signs of activity and inflammation are much more commonly seen than at other times of the year, and during the months of February to May the proportion of such lesions showing bacilli in smears rises from a normal of about 6% to over 20%, falling suddenly to below 5% in June. In many cases activity of leprosy lesions is almost confined to a certain season and if it can be prevented the prognosis may be much improved. The cause of these seasonal variations is not clear but it appears probable that it is climatic conditions.

(b) Complement fixation in leprosy.

A thorough study of the complement fixation test with W.K.K. antigen has been carried out. Several hundred tests have been performed. The test gives positive results in practically all cases of leprosy in which bacilli can be found in smears. Bacteriological examination is an easier procedure so that the W. K. K. test is of little value in diagnosis or prognosis. Studies of complement fixation with antigens prepared from other acid-fast bacilli are being carried out.

(c) Culture work.

The trial of Llera's method of cultivating lepra bacilli in the blood has given negative results.

An acid-fast bacillus has been isolated from rat leprosy material by tissue culture methods. This organism in culture differs in morphology from the organism of rat leprosy but the morphology changes on injection into rats so that it closely resembles the rat leprosy bacillus. In this respect this organism is similar to some of the acid-fast bacilli isolated from leprous material by other workers. The bacillus is, however, not pathogenic to rats and we do not consider that it is related to the organism of rat leprosy. This experiment shows the importance of working with rat leprosy, for by inoculation we have shown that the culture is not genuine.

(d) Animal experiments.

A number of Syrian hamsters some splenectomised and some not splenectomised have been inoculated with human leprosy material in order to prove the findings of Adler that the splenectomised Syrian hamster is susceptible to human leprosy. This work is still in progress and no results are yet available.

(e) BIOCHEMICAL WORK.

The study of the blood cholesterol in leprosy has been concluded. Total plasma cholesterol has been determined in 23 non-lepers and 229 lepers (109 cutaneous and 120 neural cases). In 87 of the lepers the cholesterol-ester content of the blood has been determined in addition. The results are being analysed. On the whole the blood cholesterol content in the neural type of case appears to be much the same as in normal controls while in the lepromatous type of case it is lower.

(f) STATISTICAL SURVEY WORK.

A complete statistical survey of leprosy in a group of 42 villages have been completed and a preliminary report has been issued. The chief findings are as follows :—

The gross incidence is 4.38%. Only 18% of cases are of severe lepromatous type, a much lower figure than is found in some other parts of the world. There was an increase in incidence up to adult life but the great majority of infections were contracted early in life. Males showed a higher incidence and greater severity than in females. 80% cases of leprosy gave a history of contact. About 1/3rd were infected by near relatives, 1/3rd by distant relatives and 1/3rd by non-relatives and unknown persons. Close contacts at early ages more commonly produced the severer forms of the disease. Leprosy appeared to be transmitted by the severer lepromatous cases and not by the mild neural cases.

27. Enquiry into the influence of skimmed milk on the progress and treatment of leprosy by Dr. R. G. Cochrane, Chingleput, Madras.

The enquiry on the influence of skimmed milk and the progress of treatment of leprosy is now in its second year, and no outstanding results can be demonstrated but certain conclusions can be tentatively drawn. Only children have been selected for this experiment, and these have been divided into two groups.

(1) Children with definite lesions, but which were not considered to be serious enough to warrant treatment.

(2) Children under treatment.

All children were given 8 ozs. of fluid milk a day and the general conclusion was that it appeared that in the amount given and in the duration of time, 9 to 18 months, the administration of skimmed milk seemed to have no influence in assisting the milder lesions of leprosy to subside. The group under treatment was divided into those having neural type leprosy and those with lepromatous type leprosy. Some of these were at the Lady Willingdon leper settlement others were at the Silver Jubilee clinic for the study of childhood leprosy at Saidapet. At the Lady Willingdon leper settlement 27 cases have been on skimmed milk ; out of these 8 have become negative and 2 became worse. Of the 90 children not on skimmed milk, 11 became negative and 6 became worse. It is possible that if more cases had been placed on skimmed milk the results would have been more comparable. The lepromatous type cases in the Saidapet clinic were few but the results were interesting. Of 10 lepromatous type cases on skimmed milk 5 became negative and only one became worse. Of the 9 lepromatous type cases receiving no skimmed milk only 2 became negative and 3 became worse.

Since the writing of this report in November, more cases in the diet group at Chingleput have become negative and three in the no-diet group.

The total number of lepromatous type of cases on skimmed milk at Saidapet and Chingleput is 37 ; out of these 17 have become negative, giving a percentage of 45.94, and 4 worse, giving a percentage of 10.81. The total number of cases at Saidapet and Chingleput not on skimmed milk was 99 ; of these 16 became negative giving a percentage of 16.16 and 11 became worse giving a percentage of 11.99. As the group on no-milk is more than three times as large as that on milk no definite conclusions can be drawn, but we think that we can tentatively conclude that :—

(1). The administration of skimmed milk in the amount given and in the duration of time (9 to 18 months) seems to have no influence on the neural lesions of leprosy.

(2). That there is, however, some slight evidence that this product is of value in rendering the lepromatous type cases negative in a shorter period of time.

We have taken a fresh selection of cases in order to endeavour to confirm the finding that skimmed milk appears to hasten recovery in the lepromatous type case in children.

PLAGUE

28. Plague researches under the Director, Haffkine Institute, Bombay.

1. Plague vaccine.

During the year work on plague vaccine and antiplague serum was continued at the Haffkine Institute.

In the last report a considerable amount of work was reported on the role played in the preparation of plague vaccine by (a) the temperature of incubation of culture (27° C and 37° C), (b) the medium in which cultures are grown (broth and agar), (c) the virulence of the organism and (d) the method of killing cultures. Vaccines prepared from heat-killed virulent cultures and live avirulent were also compared. At the outset it had been decided to make four determinations of the protective power of each vaccine under study by the standardised methods described in the 1932-35 Report. But at the time of reporting, in a number of instances, four determinations had not been completed. During the year work was continued to complete four determinations for each vaccine. Considerable difficulty in correctly evaluating the results of these measurements had been experienced in previous years. The statistical method described in the Medical Research Council Report No. 183, 1937, and recommended to us was found to be too time consuming for our purpose. But while writing up the results of this year's work a simpler statistical method for estimating fifty per cent. end points by Reed and Muench came to our notice (Reed, L.J. and Muench H., American Journal of Hygiene, 1938, 27, 493). We have found this method to be eminently suited for our work, and have used it for calculating the results reported below. In the case of each vaccine 100 mice were used for the four measurements carried out and five graduated doses of each vaccine were used, in the case of one vaccine, however, 250 mice were used.

A. The relative protective power of broth vaccines incubated at 27° C and those incubated at 37° C.

Broth vaccines	Minimum mouse protective dose.
Vaccine 120*/5H (15th September 1936) incubated at 27° C for four weeks (four experiments) ..	0.0025 c.c.
Vaccine 120/5H (15th September 1936) incubated at 37° C for four weeks (four experiments)	0.0080 c.c.
Vaccine I (21st October 1936) incubated at 27° C for four weeks (four experiments)	0.0021 c.c.
Vaccine I (21st October 1936) incubated at 37° C for four weeks (four experiments)	0.0043 c.c.

In all cases growths were killed at 55° C for 15 minutes, and carbolised to contain 0.5 per cent. phenol.

B. The relative protective power of agar vaccines incubated at 27° C and those incubated at 37° C.

For these experiments a 48-hour broth culture of a given strain was planted on nutrient agar slopes. These were incubated for 48 hours some at 27° C and others at 37° C. Suspensions were then made with saline and standardised to contain 1,500 million organisms per c.c. Growths were killed at 55° C for 15 minutes and then phenol added (0.5 per cent.). The number of 1,500 million organisms was fixed by actual experiment, so that the best agar vaccine may have about the same protective power as the best broth vaccine.

Agar vaccine	Minimum mouse protective dose.
Vaccine 120/5H (24th August 1936) incubated at 27° C (4 experiments)	0.0851 c.c.
Vaccine 120/5H (24th August 1936) incubated at 37° C (10 experiments)	0.0017 c.c.
Vaccine I (26th August 1936) incubated at 27° C (4 experiments)	0.0590 c.c.
Vaccine I (26th August 1936) incubated at 37° C (4 experiments)	0.0033 c.c.

C. Live avirulent broth vaccines compared with heat-killed broth vaccines.

In the previous report we had described a method of maintaining the virulence of plague cultures for long periods, and also a method for making cultures avirulent. We were thus enabled to have the same strain both in a fully virulent and in an avirulent form. Using the virulent and the avirulent forms of strain 120/5H we have made broth vaccines, incubated for 4 weeks. Temperatures of incubation employed were 27° C and 37° C. The vaccines made from virulent strains were first killed at 55° C for 15 minutes and then phenol added (0.5 per cent.)

* This strain through typographical error was designated as 125H in the last report. The correct number is 120/5H.

Broth vaccines.	Minimum mouse protective dose.
Heat-killed vaccine 120/5H (15th September 1936) incubated for 4 weeks at 27° C (4 experiments) ..	0.0025 c.c.
Live avirulent vaccine 120/5H (26th September 1936 and subsequent dates) incubated for 4 weeks at 27° C (4 experiments)	0.0096 c.c.
Heat-killed vaccine 120/5H (15th September 1936) incubated for 4 weeks at 37° C (4 experiments) ..	0.0080 c.c.
Live avirulent vaccine 120/5H (26th September 1936) incubated for 4 weeks (4 experiments)	0.0038 c.c.

D. Live avirulent agar vaccines compared with heat-killed agar vaccines.

The strains used in the experiments detailed above under C were used in this experiment. The cultures were grown for 48 hours on nutrient agar slopes and suspensions made in saline. These were standardised by colony counts to contain 1,500 million organisms per c.c. The virulent cultures were killed at 55° C for 15 minutes and phenol added (0.5 per cent.).

Agar vaccines	Minimum mouse protective dose.
Heat-killed vaccine 120/5H (24th August 1936) grown at 27° C (4 experiments)	0.00851 c.c.
Live avirulent vaccine 120/5H (14th April 1937 and subsequent dates) grown at 27° C (4 experiments) ..	0.1175 c.c.
Heat-killed vaccine 120/5H (24th August 1936) grown at 37° C (10 experiments)	0.0017 c.c.
Live avirulent vaccine 120/5H (12th December 1936) grown at 37° C (4 experiments) ..	0.0008 c. c.

E. Effect of the method of killing cultures on the potency of the resultant vaccine.

Broth vaccines	Minimum mouse protective dose.
Incubated at 27° C for 4 weeks—	
Vaccine 120/5H (15th September 1936) killed at 55° C for 15 minutes and then added phenol 0.5 per cent. (4 experiments)	0.0025 c.c.
Vaccine 120/5H (15th September 1936) killed at 60° C for 1 hour and then added phenol, 0.5 per cent. (4 experiments)	0.0097 c.c.
Vaccine 120/5H (15th September 1936) killed by the addition of phenol, 0.5 per cent., no heat applied (4 experiments)	0.0028 c.c.

Vaccine 125/5H (15th September 1936) killed by the addition of formalin 0.025 per cent., no heat applied (4 experiments)	0.0046 c.c.
Incubated at 37° C for 4 weeks—	
Vaccine 120/5H (15th September 1936) killed at 55° C. for 15 minutes and then added phenol 0.5 per cent. (4 experiments)	0.0080 c.c.
Vaccine 120/5H (15th September 1936) killed at 60° C for 1 hour and then added phenol, 0.5 per cent. (4 experiments)	0.0159 c.c.
Vaccine 120/5H (15th September 1936), killed by the addition of formalin, 0.025 per cent. (4 experiments) ..	0.0093 c.c.
Incubated at 27° C for 48 hours—	
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c. killed at 55° C for 15 minutes and then added phenol, 0.5 per cent. (4 experiments)	0.0850 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed at 60° C for 1 hour and then added phenol 0.5 per cent. (4 experiments)	0.3000 c.c.

Broth vaccines	Minimum mouse protective does.
Vaccine 125/5H (24th August 1936), suspension contained 1,500 million organisms per c.c. killed by the addition of phenol 0.5 per cent. no heat applied (4 experiments)	0.0650 c.c.
Incubated at 37° C for 48 hours—	
Vaccine 120/5H (24th August 1936), suspension contained 1500 million organisms per c.c., killed at 55° C for 15 minutes and then added phenol, 0.5 per cent. (10 experiments)	0.0017 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c., killed at 60° C for 1 hour and then added phenol, 0.5 per cent. (4 experiments)	0.0016 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c. killed by the addition of formalin 0.025 per cent., no heat applied (4 experiments)	0.0019 c.c.
Vaccine 120/5H (24th August 1936), suspension contained 1,500 million organisms per c.c. killed by the addition of phenol, 0.5 per cent. (4 experiments)	0.0020 c.c.

F. Vaccines made from different avirulent strains compared.

Last year we had worked with strain 120/5H made avirulent by repeated (64) weekly subcultures at 37° C. During the year we had made

strain I avirulent. This strain had needed 80 weekly subcultures to make it avirulent. 75th subcultures still showed a little but measurable virulence. From this avirulent strain we have made several broth and agar vaccines and have tried to determine the minimum mouse protective dose. In the case of three agar vaccines incubated at 37° C we have given as much as 0.55 c.c. per mouse and even this dose failed to protect. In the case of broth vaccine incubated at 27° C as much as 0.8 c.c. failed to show any protection, while in the case of 120/5H strain as little as 0.0008 c.c. of 37° C agar vaccine and 0.0096 c.c. in the cases 27° C broth vaccine were found to be the minimum mouse protective dose.

This observation is interesting. Both the strains 120/5H and I were fully virulent to start with and they were both made avirulent by the same method. Yet the behaviour of the two resulting avirulent strain is totally different; strain 120/5H has retained its protective properties, while strain I seems to have lost its antigen value altogether. We propose to study this problem further. We have made two more strains avirulent for this study.

II. Antiplague serum.

During the period under report an opportunity occurred for testing the value of the plague anti-serum in the field at Bettiah, Bihar. At the beginning of this year a small epidemic of plague occurred at Bettiah. Bettiah Raj and Bihar Government through their Director of Public Health offered the Institute the necessary facilities for the trial. A temporary hospital was erected for the purpose and other assistance given. For all this our thanks are due to Bettiah Raj, Bihar Government and their Director of Public Health.

The trial lasted from the middle of January to the end of April. In all 139 cases were admitted to the hospital out of which 15 cases proved to be not cases of plague. Leaving out of consideration these 15 cases, there were thus 124 cases of plague.

Alternate admission was taken for treatment with serum. No selection of any kind was made. The cases, not given serum, were treated with iodine solution given intravenously, and were considered as controls. Of the total number of 124 cases, 69 were treated with serum. Of these 19 died giving a percentage mortality of 27%. Of the 55 controls, 36 died, giving a percentage mortality of 65%. The excess in the number of cases treated over those used as controls, in spite of taking alternate cases, was due to the fact that during the third week of the trial all cases admitted were treated for reasons over which we had no control.

The following analysis of the cases gives a better idea of the value of the serum :—

Classification of cases.	Treated with serum.		Controls	
	Number.	Deaths	Number.	Deaths.
Bubonic	34	1*	16	2
Mild septicaemia (up-to 40 colonies per 0.25 c.c. of blood) ..	15	4	15	11
Severe septicaemia (more than 40 colonies per 0.25 c.c. of blood) ..	20	14	14	23
Total	69	19	55	36

* This patient died of lobar pneumonia.

It will be noticed that the classification employed this time is somewhat different from that used on a previous occasion. It was felt the previous classification did not represent the correct differences in severity among different cases.

III. STUDY OF ANTIGENIC STRUCTURE OF PAST.PESTIS.

This enquiry was started in April of this year and after spending a few weeks in getting the necessary equipment, investigation of the antigenic structure of Past.pestis has been undertaken along the following lines :—

(1) Isolation of an active protein fraction of immunising value in the Haffkine vaccine is being made by saturating the supernatant fluid with sodium sulphate. The precipitate is dissolved and reprecipitated with various concentrations of sodium sulphate. The products obtained are further purified and dialysed to free them from the salt. Their serological activity as well as their immunising capacity against Past.pestis will be tested.

(2) Recently nucleoproteins are assuming a great importance in the study of the antigenic structure of micro-organisms. So attempts are being made to isolate any nucleoprotein fraction present in the supernatant fluid by precipitating it with dilute hydrochloric acid in cold. This is followed by further precipitations with dilute HCl from alcoholic solutions (50%) of the precipitate. A substance has been obtained that reacts with the antiserum and gives very strong tests for protein. Further investigations are being made on the preparation and properties of this substance.

(3) Isolation of an active polysaccharide fraction from the supernatant of the Haffkine vaccine. The supernatant is concentrated in vacuo at a bath temperature of about 40° C and the concentrate is precipitated with alcohol in the presence of sodium acetate. This precipitate is further purified from protein and inorganic impurities by repeated precipitations with alcohol in the presence of acetic acid and sodium acetate, and shaking a few times with chloroform and n-Butyl alcohol. The final product gives negative test for protein and a strong Molisch test for carbohydrates. It reacts with horse as well as rabbit antisera for Past.pestis.

Further experiments are being made on the properties of this product, which is presumably a carbohydrate.

(4) Besides the above a quantitative precipitin test is being developed to evaluate the potency of the antiserum obtained from horses. The supernatant of the Haffkine vaccine is being used as the antigen. The results obtained show that there is a certain degree of correlation between the precipitin content and the results of the mouse protection test, but much more work is needed to elucidate this point further.

29. Enquiry into the recrudescence of plague in the Cumbum Valley by Dr. P. V. George, Research Health Officer, Madras.

Nineteen villages and hamlets around Cumbum town were fumigated during the year. Six of these localities were fumigated on threat of plague, two of which have since been free from plague for over an year, three for 8 to 10 months, and one for 2 months. Infected villages have yielded varying results, depending on the severity of rat plague and the intensity of

work. If it were possible to fumigate these villages every quarter consistently for a few years, there is a reasonable prospect of success in eradicating endemic plague in this valley.

1. Two lakhs five thousand nine hundred and ninetyeight rat holes and 6,372 cracks in walls in 25,471 houses were fumigated during the year. 5,963 R. rattus, 284 bandicoots, 8,876 house mice, 96 musk rats and 22 snakes were picked up dead or dying during these operations. Eleven thousand seven hundred and eighty-six rodents were examined in the laboratory and 42 proved positive to plague. 98 carcasses from various villages were examined and 57 were positive. Using bone marrow material for culture and animal inoculation, it has been possible to diagnose infection in some specimens till 102 hours after death.

2. The problem of killing rats and fleas in thatch roofs was specially studied during the year. The usual type of mud houses in the Cumbum Valley can in no sense be rendered gas-tight, but for purposes of killing rats and fleas, it is found enough if the doors and windows are tightly closed, all small openings packed with wet mud and any gap between wall and roof stuffed with gunny rolls. When the gas is pumped in and it diffuses through the thatch, all rats and fleas therein are immediately killed. A concentration of 3 to 4 ozs. of HCN (12 to 16 ozs. of cyanogas) per 1000 c. ft. and an exposure of 3 to 4 hours are found sufficient for this purpose.

3. Rat flea surveys of selected villages immediately before and after fumigation of rat holes have shown that while rat densities were reduced by 40 to 80%, the flea indices showed no marked difference, possibly due to concentration of other wild fleas on the surviving rats.

Rat nests are admittedly the prolific nurseries of fleas but for different species of fleas certain preferences are noted according to the location of the nest and the material used for its construction. While astia fleas predominate in nests recovered from underground rat holes, cheopis shows no such preference at all. Admixture of cotton in the rat nests effects a clear relative increase in cheopis fleas. Bandicoot nests yielded about 93% astia and 7% cheopis fleas. The nests of wild rodents, such as gerbils, yielded a cent per cent. astia count.

Fumigation of rat nests in air-tight chambers effects thorough destruction from eggs to imago of fleas. Rat nests recovered from fumigated rat holes have been observed to yield adult fleas after sufficient incubation, showing thereby that the eggs have not been affected.

4. Cyanogas fumigation was adopted as a routine measure of disinfestation in the control of grain traffic etc., from plague affected villages. The traffic is largely in bullock carts and to a small extent in motor lorries and all varieties of local produce and personal effects are encountered. Fumigation is done under oiled tarpaulins over a prepared ground on the roadside, with a shed for protection against weather. Concentrations and exposure periods as mentioned in the report for 1937 were adopted. This work was under the direct control of the District Plague staff though in consultation with the Research Unit at Cumbum. It is noted that the best procedure will be to fumigate the loaded vehicles in toto under suitably sized tarpaulins.

5. In two simultaneously infected villages, cyanogas in one, and cymag in the other, (a product of the Imperial Chemical Industries Ltd.) were employed for fumigation of rat burrows, to compare their comparative efficiency. Accidentally perhaps, the village where cymag was employed

had a severer epidemic. Cymag evolves HCN very slowly and thus permits many more rats to escape than with most other cyanide products. It is white in colour when fresh and shows no characteristic change on deterioration. With cyanogas or calcid, one can easily say by colour whether the stuff is inert or potent. Further, cymag has a tendency to form lumps and to stick to the sides of the container and hose of the pump with the risk of sometimes choking the pump.

6. A hut almost like the usual type of huts in Cumbum Valley has been constructed, introducing certain devices to eliminate rat harbourages. Observations as to the efficiency of the devices employed are in progress.

RABIES.

30. Antirabic vaccine enquiry by the Director, Pasteur Institute of India, Kasauli.

I. Human experiments.

Experiments to test the value of immune serum in antirabic treatment.

The investigation into the value of antirabic serum as an adjunct to treatment with a carbolized dead vaccine was continued. The results from the commencement of the investigation are given below :—

(1) Treated at the Pasteur Institute, Kasauli.

Total treated with serum plus vaccine—1405 cases.
Deaths—23. Death rate—1.64%
Total controls treated with vaccine alone—716 cases.
Deaths—17. Death rate—2.37%.

(2) Treated at selected centres in the Punjab and the United Provinces.

Total treated with serum plus vaccine—2,234 cases.
Deaths—40. Death rate—1.79%.
Total controls treated with vaccine alone—4,971 cases.
Deaths—62. Death rate—1.25%.

The Institute results by themselves show a slight advantage in the use of serum, but not those of the centres.

II. Animal experiments.

Experiments connected with the use of antirabic serum.

Two experiments were conducted to determine the potency of the sera of hyperimmunized horses by testing their neutralizing action on rabies fixed virus. Rabbits were used as the experimental animals.

Dilutions of serum-virus mixture were prepared by mixing a 4% emulsion of fixed virus and the sera of Horses I, II, III, IV and a normal horse separately. Dilutions were also made separately with 50% serum and fixed virus.

The serum-virus mixtures were incubated at 37° C. for periods varying from 1 to 2 hours, and at the end of that time two rabbits were passaged through the cisterna magna route with each serum-virus mixture. The test dose was 0.2 c.c.

As a control for the fixed virus used in the experiment one rabbit was passaged with fixed virus only. The results are given in tabular form :—

Dilution of serum virus mixture.	Horse I.		Horse II.		Horse III.		Horse IV.		Normal horse.	
	Passaged.	Survived.	Passaged.	Survived.	Passaged.	Survived.	Passaged.	Survived.	Passaged.	Survived.
Virus 1 : 50 Serum 1 : 2	2	2	2	1	2	0	2	0	3	0
Virus 1 : 50 Serum 1 : 4	2	1	2	0	2	0	2	0	0	0

The rabicidal value of the serum of Horse I was very satisfactory. That of Horse II poor, while the sera of Horses III and IV was completely devoid of viricidal properties.

(B) Two experiments were undertaken :—

(1) To compare the relative viricidal values of horse and sheep antirabic serum.

(2) To determine the mode of action of antirabic serum.

Sera from Horses I and II which had been immunized for 3 years but which did not give a positive complement fixation test, and the sera from two sheep, also highly immunized but which gave a strongly positive complement fixation test, were examined as follows :—

Serum-virus mixtures were prepared as before and two rabbits were inoculated with each mixture of undiluted serum and virus and one each with the diluted serum and virus.

For the second part of the experiment 1 c.c. each of the serum-virus mixture containing a 1 in 2 dilution of serum in a 1 in 50 suspension of fixed virus from Horse II and sheep I were put into sterile ampoules and stored in the ice-chest after 2 hours incubation at 37° C. At the end of 24 and 48 hours respectively part of the above two mixtures were washed in normal saline by centrifuging several times and the deposit collected and diluted with distilled water before use. Two guinea-pigs were inoculated with the washed suspension and one each with the unwashed serum-virus mixture.

The result is given below in tabular form for part I of the experiment :—

Dilution of serum-virus mixture.	Horse I.		Horse II.		Sheep I.		Sheep 40.	
	Passaged.	Survived.	Passaged.	Survived.	Passaged.	Survived.	Passaged.	Survived.
Virus 1 : 50 Serum 1 : 2	2	1	2	1	2	0	2	2
Virus 1 : 50 Serum 1 : 4	1	0	1	0	1	1	1	1

The two guineapigs in part II of the experiment died of other causes. All the others survived.

It would appear that

(1) The complement fixation test is not always a reliable indication of a potent serum.

(2) Fixed virus is destroyed by antiviral serum *in vitro*.

(C) Eight rabbits from the two previous experiments which had survived a dose of serum-virus were given a test dose of 0.2 c.c. of a 1 in 10,000 suspension of fixed virus to determine whether they had developed any immunity. Seven died of fixed virus rabies, and one due to other causes.

The results indicate that fixed virus is killed by the antiviral serum and no immunity is produced by the subdural inoculation of such a mixture against a subsequent infection.

(D) Two experiments were carried out to determine whether any protective value is afforded by the use of antirabic serum given intrathecally as a prophylactic measure.

Rabbits were anesthetized and one c.c. of C. S. F. removed by cisterna puncture from each animal, and replaced in six rabbits by a c.c. each of immune horse serum and in six others by a c.c. each of immune sheep serum.

The first batch of six rabbits was infected after a week with 0.2 c.c. of a 1 in 10,000 dilution fixed virus subdurally and the second batch with 1 c.c. of a 1% emulsion of street virus in the muscles of the neck after the same interval.

The first batch of six rabbits died of fixed virus rabies. Of the second batch infected with street virus, only one has survived. Serum given intrathecally as a prophylactic measure does not appear to afford any protection.

Experiments in connection with the duration of immunity.

To determine the duration of immunity in animals after a course of antirabic inoculations, monkeys which survived from various experiments involving a course of antirabic vaccine were used. Period of $7\frac{1}{2}$, $8\frac{1}{2}$ and $9\frac{1}{2}$ months after treatment were tested.

None of the treated animals which were tested for immunity developed rabies. 33 $\frac{1}{3}$% of the controls used to check the virulence of the street virus, developed rabies.

The street virus was obviously a weak one and the result therefore may be considered only fair.

Experiments in connection with the antigenic value of monkey brain antirabic vaccine.

An experiment to compare the preinfectional immunizing value of formalized 5% Paris sheep and Paris monkey brain vaccine against carbolized 5% Paris sheep and Paris monkey brain vaccines was undertaken to complete the investigation of the claim by Van Stockum that a monkey brain vaccine was "par excellence" the antigen of choice in antirabic treatment.

Forty monkeys were divided into four groups of 10 each and treated as under :—

Group I with formalized monkey brain vaccine.
,, II ,, ,, sheep ,, ,,
,, III ,, carbolized monkey ,, ,,
,, IV ,, ,, sheep ,, ,,

The immunizing dose was 2 c.c. given daily for 14 days.

About six weeks after completion of treatment, the animals in the four groups together with 10 untreated controls to check the virulence of the infecting street virus were infected with 2 c.c. of a 2% emulsion of street virus in the muscles of the neck. The result is given in tabular form :—

Nature of treatment	No. of monkeys infected	Died of other causes	Died of rabies	Percentage deaths from rabies excluding deaths from other causes.
Formalized monkey brain vaccine	9	—	—	0
Formalized sheep brain vaccine	7	—	2	28.6
Carbolized monkey brain vaccine	5	—	—	0
Carbolized sheep brain vaccine	6	—	—	0
Untreated controls . .	10	1	8	88.9

The experiment was undertaken during the wet weather and mortality from dysentery reduced the number of animals in the treated groups. The result indicates that carbolized monkey brain vaccine is not superior to that of a carbolized sheep vaccine.

Experiments in connection with the therapeutic value of animal vaccine.

An experiment was undertaken to compare the preinfectional immunizing value of carbolized 6% Paris sheep vaccine manufactured by this Institute for the treatment of animals against that of a proprietary vaccine which is on the Indian market.

Dogs and monkeys were used.

Five dogs and five monkeys were treated with the Institute vaccine for 7 days.

On the last day of the above treatment, a second group of 5 dogs and 5 monkeys were inoculated with one dose of the proprietary vaccine. The dosage was that recommended by the manufacturers for prophylactic immunization.

After completion of treatment the animals in both groups, together with a third group of controls (4 dogs and 5 monkeys) were infected with street virus. The infecting dose was 2 c.c. of a 2% emulsion. The result is tabulated :—

Treatment.	Dogs.			Monkeys.			Remarks.
	Infected.	Died of other causes	Died of rabies	Infected.	Died of other causes	Died of rabies	
Institute vaccine	4	1	—	3	—	—	One dog in each group died of other causes before infection.
Proprietary ,,	4	1	3	5	—	3	Two monkeys in the animal vaccine group died of dysentery before infection.
Controls	4	—	4	5	—	2	

The Institute vaccine affords considerable protection while the proprietary vaccine used as a "one shot" dose was found not to be satisfactory against the Indian strains of rabies street virus.

Experiments in connection with improvements in animal vaccine.

The preinfectional immunizing value of carbolized 5% saline vaccine was compared with (a) a carbolized 5% distilled water vaccine and (b) the filtrate from that vaccine.

The saline vaccine was identical with that in general use. The distilled water vaccine was made up in the same way except that carbolized distilled water was used. Both vaccines were proved sterile and stored at room temperature for about one month. At the end of this period sufficient sodium chloride was added to the distilled water vaccine to convert it into a saline vaccine. It was hoped by this means to ensure maximum rupture of the brain cells and nuclei in distilled water before the necessary salt was added.

A portion of the distilled water vaccine was filtered and the filtrate used for experiment.

Thirty monkeys were inoculated as under :—

Group I —2 c.c. saline vaccine daily for 14 days.

,, II —2 c.c. distilled water vaccine daily for 14 days.

,, III —2 c.c. filtrate from distilled water vaccine daily for 14 days.

About two months after completion of treatment these animals together with 10 untreated controls were infected with 2 c.c. of a 2% emulsion of street virus from a first passage guineapig in the muscles of the neck.

Nature of treatment.	No. of monkeys infected	Died of other causes.	Died of Rabies	Percentage from rabies excluding deaths from other causes
Saline vaccine ..	9	—	1	11.1
Distilled water vaccine ..	10	—	1	10
Filtrate from distilled water vaccine	10	—	2	20
Controls	10	—	10	100

The result suggests that a filtrate from a 5% vaccine prepared with distilled water compares favourably with that from a saline vaccine but further experiments will be necessary before any conclusion can be arrived at.

Culture of rabies fixed virus.

Attempts to culture fixed virus rabies on embryonic chick brain met with no success.

TUBERCULOSIS.

31. Tuberculosis enquiry by Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta.

I. TUBERCULOSIS IN RELATION TO INDUSTRY.

This pioneer investigation is being continued at the Angus Jute Mills, 23 miles from Calcutta, which employ 5,000 labourers from various parts of India. It is fortunate for the investigation that the mill possesses a good laboratory and a useful X-ray plant, which are being fully made use of.

(*a*) *Intensive study of sample workers from different departments.*

Two hundred and sixty three workers were selected as samples from various departments of the mill (ten in number) who were put through a thorough examination including physical examination, sputum and blood tests and chest skiagraphy. The previous life of the worker, history of previous illness and of contact with tubercular cases, the details of industrial life with age at entry, duration and nature of work, his financial status, housing and other relevant points were recorded. These 263 cases were distributed as follows :—Batching section—82, Preparing section—18, Spinning—63, Copewinding—16, Beaming—10, Weaving—42, Finish ing—10, Babus (clerks) —4, Mistries—8 and Europeans—10.

Previous habitation :—80% from rural, 18% from semi-rural and only 2% from urban areas.

Duration of industrial life:—Below 5 years—9%, below 10 years—19%, below 20 years—45%, below 40 years—25% and above 40 years —2%.

Physical and radiological examinations:—1.9% of these workers were labelled as "definite" cases (i.e. both sputum positive and radiologically active infiltration in lung parenchyma) of pulmonary tuberculosis, 5.3% as "possible" cases (i.e. radiological findings indicative of active disease but sputum either not available or negative) and 59% as "arrested" or "healed" lesions either in tracheo-broncho-pulmonary glands or in lung parenchyma.

Exaggerated bronchial striations were present in skiagraph in 75% and arborization with small mottlings in 27% cases. (In assessing chest skiagraphs, Cummins' modification of Irvine's classification and Cummins' method of assessing the extent, density and general character of lesions were followed.)

Thirty-two per cent. of these selected cases showed clinical evidence of bronchitis. Allergic (?) skin manifestations (eczema) were present in 4%.

Blood tests.—Von Bonsdorff's count and lympho-monocyte ratio tests were done in 225 cases. Pathological deviation (i.e. V. B. below 200 and monocytes over 15) was present in 7.6% cases. A milder deviation, as indicated by V. B. count between 200-250, was present in 54.3% cases and by lympho : monocyte ratio with monocytes over 15 in 11.8% cases and over 10 in 19% cases. Blood tests could be done in 4 out of the 5 "definite" cases-of these three showed definite pathological deviation by *both* tests and one was normal. Among the 8 "probable" cases whose blood was examined none showed definite pathological deviation by *both* tests, one indicated it by V.B. and 4 by lympho-monocyte ratio. A considerable degree of eosinophilia was found by the differential count of leucocytes in blood—7% showed eosinophilia over 15%, 21% between 10-15%, 32% between 5-10%, and 40% less than 5%.

(*b*) *Serial examination and graded tuberculin testing of all workers.*

Having had an idea of the position in different departments, a serial tuberculin testing by 1 in 1000 dilution of Mantoux test was commenced. The "batching", "preparing" and "spinning" departments have been taken up first and, up to the time of writing the report, 1,360 workers have been tested and examined in a way similar to what had been done for the sample investigations described above, except radiologically which is being done only in suspected cases and in cases showing strong tuberculin reactions. Out of 400 cases of strong tuberculin reactors and clinically suspected cases to be X-rayed, it has been done only in 91 cases thus far, among whom 9 have proved to be X-ray "definite" and 8 "probable" ones.

Results of tuberculin test—Positive=86.5%

(+ = 35.3%, + + = 24.6%, + + + = 21.6% and + + + + = 5.1%)
Physical findings suggestive of pulmonary tuberculosis were found in 48 cases. Both exaggerated tuberculin reaction and suspicious physical findings were detected in 12 persons. Bronchitis was detected in 224, Jute eczema in 126 and naso-pharyngeal catarrh in 49 persons.

A history of pneumonia (?) was given by 15 workers previous to their entry and by 58 workers after their entry into this industry.

(*c*) *Home survey.*

Five hundred and three dwellings in barracks have hitherto been investigated. This included a survey of the house and its surroundings, the social customs and habits, income general mortality and tuberculosis morbidity and mortality. The data are being recorded in specially prepared schedules.

It has been generally noticed that rooms, which had seldom cross ventilation, were very overcrowded, general sanitation was bad, many customs prevailed which were likely to favour spread of infection, and that the diet was inadequate and badly balanced. A considerable number of workers are addicted to alcohol and other narcotics. The average weekly income of workers is Rs. 2-5-0, whereas total debt per head is Rs. 30/- for which they have often to pay a very high interest (6 pies per rupee per week). The children of these workers looked ill-nourished and a fair number showed palpable cervical glands (not yet critically surveyed).

(*d*) *Contact survey.*

Twelve houses, belonging to 3 " definite " and 9 "probable" cases, of which 4 are located in a neighbouring village, have

hitherto been surveyed. Among the contacts 13 were children and 27 adults. Of the 13 children, all of whom gave a positive reaction, 11 gave a + + + reaction and 2 + + reaction. Of 27 adults, all were positive, two showing + + + + reaction, one + + reaction and the rest + reaction. One adult has shown clinical signs of disease but he and the other contacts with strong reactions are awaiting X-ray investigation.

(e) Dust hazards in the industry.

The different processes through which the jute fibre passes in order to be finally baled emit a large amount of dust. In order to determine the size and concentration of suspended dust particles, 144 samples from various departments have been examined by Bausch and Lomb dust counter. A majority of the dust particles examined were below 5 microns in size (about 90% of jute particles were between 1-3 microns).

It is already known that dust particles bigger than 10 microns can not enter the pulmonary alveoli and that unaided vision can only see particles bigger than 60 microns. The most dangerous size is between 0.5 - 5 microns. The concentration of dust is considered harmful if it exceeds 5,000,000 particles per cubic foot. The dust concentration at many places in the mill, especially in the "breaking line" (a part of "preparing" section), and in "spinning" and "selecting" (a section of "batching" department) departments far exceeds 5 million particles per cubic foot (a majority between 7-10 millions).

At first sight it seems that such dust conditions would produce harmful effects by causing some amount of disability on the health of the workers, but it is remarkable that no case of physical disability, as evidenced by shortness of breath or disabling recurrent chest complaints, could be detected. Chemical analysis has shown that the jute fibre consists mainly of resistant cellulose, hemi-cellulose and lignin. A very minute quantity of silica is also present in the ash. The inert nature of the chemical structure of the fibres probably accounts for no great disabling effect. Although no disability has been detected, the high concentration of dust is probably responsible for the large amount of bronchitis (as evidenced by diminished chest expansion and frequently exaggerated bronchial striations on the skiagraph) noticed.

II. TUBERCULOSIS IN "HOME CONTACTS".

Two hundred and twenty-one children below 15 years have been examined and followed up since last year, bringing the total to 838. Owing to the difficulty of a home survey of scattered houses and of bringing the contacts for X-ray and other investigations and to certain unavoidable difficulties about X-ray service in the hospital, the progress has not been as rapid as expected. It is proposed to raise the number of completely examined cases to 1,000 before final deductions are drawn. As soon as the tubercular homes are surveyed, it is proposed to include a certain number of non-tubercular homes, if possible, for a comparative study. A brief review of the 838 contact children examined is given below.

Of these 838 children under investigation, Mantoux test (1 in 1,000 dilution) has been done in 763 and radiological examination in 639 cases. Both Mantoux test and X-ray examination have been completed in 564 cases. 649 children were in contact with 'open' cases, 84 with 'closed' cases and 105 with 'unknown sputum' cases. The last category included cases which were X-ray positive though the sputum has not been examined, and cases which had very suggestive history of the disease

without either sputum or X-ray examination. Seven hundred and fifty children had "intimate" and 88 "distant" contact. Mantoux test was positive in 69% (61% in "closed" case contacts, 69% in "open" case contacts and 73% in "unknown sputum" case contacts). Active tubercular pulmonary lesions were detected in 11.1 cases (7.4% in "closed" case contacts, 9.2% in "open" case contacts and 23.8% in "unknown sputum" case contacts). Primary complex was noted on skiagraph in 2.3%, Healed lesions in 4.2% and definitely enlarged hilar glands in 29.8%. The difference in findings between the different categories of contacts is noteworthy but it is preferred not to offer an explanation now in view of the smallness of numbers under two out of the three categories. 47.5% of cases showed radiologically demonstrable lesions.

"Intimate" contact showed a much higher number of reactors and cases with active parenchymal lesions in the "open source" contact series than in "distant" contact. The percentage of reactors in this "open" group increased while that of active lesions decreased in proportion to the length of period lapsing after the initial contact. The percentage of positive reactors was 88% in "active lesion" series, 86% in "arrested lesion" series and 72% in tracheo-bronchial adenopathy series. No evidence was obtained pointing that the intensity of the tuberculin reaction varied directly with the activity of the lesion. The percentage of positive reaction was found to vary *directly* with age, while the incidence of demonstrable lesions varied *inversely* with age, i.e., the younger the age the stronger was the probability of tubercular lesions being present. The incidence of "active" parenchymal lesions increased with age up to 10 years after which it began to decline rapidly, while the incidence of "arrested" parenchymal lesions increased with age. The incidence of both "active" and "arrested" lesions in the "open source" series almost equalled that of the whole group. The incidence of infection, as detected by Mantoux test, was found to be 21% higher than that detected by radiography.

It has been noticed that the percentage of positive reactors and of detectable X-ray lesions increases with the prolongation of the duration of contact with "open" cases. It has also been noticed that the maximum incidence of tracheo-bronchial adenopathy (as seen in chest skiagraph) occurred after 3-4 years' contact with "open" cases and in the 5-10 year age period. The highest incidence of positive tuberculin reaction among them also occurred in the same age period.

In all, 110 tubercular homes, containing 319 children have been surveyed, of which 94 contained "open" pulmonary tuberculosis cases. Sanitary condition was bad in 56% of the houses and 39% of the houses were one-roomed tenements. 49% of the houses contained inmates with a low economic status (average income per head—less than Rs. 5/- per month). 56.7% of the contact children lived in houses with bad hygienic conditions and 36.6% in one roomed tenements. 196 out of 319 children slept in the same room with the patient (who was the source of contagion). As a majority of the cases belonged to the poorer classes, no significant correlation could be found between the incidence of infection, morbidity rate and economic status.

III. Typing of tubercle bacilli from pathological materials.

Out of 220 strains isolated, 200 strains have been typed out. Of the strains typed out, *all of which have proved to belong to the "human" type*, 126 have been obtained from lymphatic glands (cervical—68, tracheo-bronchial—37, axillary—7, inguinal—1, mesenteric—13), 2 from tonsils,

22 from cold abscess pus, 2 from urine, 4 from skin, 1 from stool, 1 from stomach wash, 35 from pleural fluid, 3 from sputum and 3 from lungs. 20 strains are awaiting typing. This line of work is being carried out in a mutilated form, the enquiry being only concerned with the study of strains obtained from infants and children and from skin and other rare non-pulmonary sources. Indian data on them are still lacking.

Out of a total of 159 non-pulmonary sources from which the strains were obtained, the age incidence was as follows :—0-2 years—6 or 4%, 2-5 years—4 or 2.5%, 5-10 years—8 or 5%, 10-15 years—13 or 8%, 15-20 years—24 or 15%, 20-25 years—30 or 18.5%, 25-30 years—22 or 14%, 30-40 years—26 or 16%, 40-50 years—9 or 6%, and above 50 years—17 or 11%.

32. Enquiry into the incidence of positive Mantoux reactors and latent tuberculosis as judged by skiagrams in medical students and nurses by Major G. F. Taylor, I.M.S., at the K. E. Medical College, Lahore.

The object of the work was :—

1. To find the incidence of negative and positive Mantoux reactors amongst medical students and nurses of King Edward Medical College, Lahore, and so estimate the number who have been infected with tuberculosis, without showing clinical disease.

2. By X-ray examination of the chest to find the number of students and nurses who have, or have had, infection of the lung with tuberculosis of the adult type without showing other signs of active disease, i.e., "latent tuberculosis".

3. To observe, over a number of years, the changes in these skin reactions and skiagrams. Medical students are almost ideal for this investigation with their five to six years course of study.

4. A number of side issues were raised, such as the percentage of Mantoux positive reactors amongst the hospital in-patients, school children and others, and among family contacts of active tuberculous cases. The normal haemoglobin percentage of the normal Punjabi was investigated and some interesting observations made on his teeth.

The first part of the work dealing with the Mantoux tests among medical students and nurses and contacts was done using the technique advised by the Tuberculosis Survey Committee of the Indian Research Fund Association.

The following results were obtained :—

I. Medical students, King Edward Medical College, Lahore.

Nationality :—Hindus, Muslims and Sikhs.

	Total.	Mantoux positive.	Mantoux negative.
1st year students	94	52 or 55 per cent.	42 or 45 per cent.
2nd ,, ,,	68	18 or 26.5 ,, ,,	50 or 73.5 ,, ,,
3rd ,, ,,	56	32 or 57 ,, ,,	24 or 43 ,, ,,
4th ,, ,,	36	29 or 80.6 ,, ,,	7 or 19.4 ,, ,,
5th ,, ,,	41	25 or 61 ,, ,,	16 or 39 ,, ,,
	295	156 or 52.9 per cent.	139 or 47.1 per cent.

The high incidence in the first year was not understood. The tests were repeated but the percentage remained twice that of the second year. Students begin their ward work in the third year. Many of the students then have frequent infection of the respiratory tract.

II. NURSES OF THE MAYO HOSPITAL, LAHORE.

Nationality :—Mostly Anglo-Indian.

Total	Mantoux Positive.	Mantoux Negative.
53	41 or 77 per cent.	12 or 23 per cent.

III. THE INCIDENCE AMONGST MEDICAL WARD CASES OF THE WEST MEDICAL WARD WAS THEN INVESTIGATED.

Nationality :—Muslim, Hindu and Sikh—lower classes.

Total	Mantoux Positive	Mantoux Negative
111	74 or 66.7 per cent.	37 or 33.3 per cent.

These tests were done on patients with chronic medical illnesses.

IV. TWO SCHOOLS AND A HINDU ORPHANAGE WERE THEN INVESTIGATED.

Cathedral High School, Lahore.

Total	Mantoux positive	Mantoux negative
120	36 or 30.0 per cent.	84 or 70.0 per cent.

Ages ranged from 9 to 16 years of age.

A lower incidence was noted in the younger children and a higher incidence in older children. Several children came from families who had had an active tuberculous case in them. All gave strong positive results. The children were of English, Anglo-Indian and Indian parents.

Sanatan Dharam High School, Lahore.

Total	Mantoux positive	Mantoux negative
44	18 or 40.9 per cent.	26 or 59.1 per cent.

Ages from 10 to 16. The children were middle-class Hindus and Muslims.

Hindu Orphanage.

Total	Mantoux positive	Mantoux negative
36	25 or 69.4 per cent.	11 or 30.6 per cent.

Ages from 10 to 18. All Hindus—Orphans.

There had been recently several cases of active tuberculosis in the orphanage.

One point of interest came out in this investigation. Tuberculin solution which had been made up for over two weeks was first used and every child gave a negative reaction. The Tuberculin solution was suspected to be stale and the tests repeated with fresh tuberculin.

FAMILY CONTACTS OF ACTIVE LUNG TUBERCULOSIS CASES.

So far, Dr. K. L. Wig, has examined six families from each of whom an active case of phthisis has been in the Mayo Hospital.

Total	Positive	Negative
45	39 or 86.7 per cent.	6 or 13.3 per cent.

Of 16 children under 10 years of age in this group of six families, there are 11 positive reactors and 5 negative, 69 per cent. positive results; one child aged 1 year gave a positive reaction. Most of the families showed all positive results. The negative reactors were, in one case, a girl who had recently married into the family, and the others very young children of one year, and three years. These figures show that, when there is an active case in the family, nearly all the members are infected with tuberculosis, without showing clinical signs. While these examinations were being done, two facts were noticed which need further elucidation and work. Firstly, a delayed positive reaction was noted in several cases, coming on several days after the injection of tuberculin. Secondly, severe extensive local reactions were noted in several cases with beginnings of necrosis and ulceration. In a few others, fever and cough came on for a day or two. More work is needed to find out the significance of these severe reactions, and the relation to recent infection with tubercle bacilli.

X-RAY EXAMINATION OF CHESTS.

There was some difficulty in deciding what was to be taken as normal and what abnormal. Advice was taken from Dr. Coombes of the Mayo Hospital and Dr. Diwan Chand Aggarwal of Lahore. During the summer of 1938, Dr. Wingfield of the Frimley Sanatorium and Brompton Hospital, London, saw the positive skiagrams and agreed with the findings.

In all 18 students out of the 400 examined show radiological evidence of healed or healing adult pulmonary tuberculosis. Skiagrams showing old calcification, typical of the primary complex of Ghon were not included. Lesions of several types were seen, early infiltration in the subclavicular areas, fibrosis, calcification, and cavitation in these areas. Several of the students came as in-patients to the wards for observation for active clinical disease. In five of them a raised sedimentation was found; several have had low fever. None of them were unable to continue their studies except for a week or two. They are being examined at intervals of one to three months and skiagrams repeated.

Out of 38 nurses examined, 2 showed positive skiagrams.

Thus 4.5% of the 400 medical students showed radiological evidence of healing or healed adult lung tuberculosis and 5.3% of 38 nurses. These cases are of the "latent" type, able to continue their work and they are hardly conscious of anything amiss.

WORK IN PROGRESS AND TO BE CONTINUED DURING 1938-39.

1. Retesting all negative Mantoux reactions of last year.
2. Repeated X-Ray examination of the positive skiagram cases in students and nurses.
3. Examination of new first year students.
4. Examination of 500 students of a Lahore non-medical college is being carried out, but the results are not ready for incorporation in this report.
5. By following up the medical history of these students and nurses during the next few years it is hoped to observe which cases develop active lung tuberculosis.

SUMMARY.

1. Mantoux positive reaction was obtained in 53% of 434 medical students at Lahore, the figure rising to 70% & 80% in the 4th & 5th year students.
2. Mantoux positive reactions were found in 77% of the 53 nurses examined.
3. Of the 400 skiagrams taken of students, 18 or 4.5% showed evidence of healing or healed adult phthisis. These cases are being observed clinically and radiologically, but as yet none of them have developed into the active "open" case with positive sputum. It is hoped to observe these "latent" cases over a number of years.

33. Tuberculin survey of school children and young adults by Major S. M. K. Mallick, I.M.S., Principal, Medical School, Amritsar.

This work was taken up as a continuation of the tuberculin survey being carried out here from a grant given by the Local Municipal Committee. A beginning had been made with the survey of the students of Amritsar, Medical School, and on the receipt of this grant the work was extended to the city; so far the work done falls in two categories :—

(1) Survey of students in different local schools and colleges.

(2) House to house survey carried out in Ward No. 9 of the city of Amritsar, which is a poor locality and as shown by the Municipality Health report tuberculosis is fairly rampant in that part of the city.

(1) So far 2451 tests have been done and the results of these tests are given below in Table No. I,

(2) House to house survey has been carried out as stated above in Ward No. 9. This is a poor Mohammedan locality where the inhabitants have to live in crowded houses under very insanitary conditions. The disease appears to be particularly prevalent in this locality. In all 462 families have been done so far and 793 individuals completely tested. Results are given in Table No. II

Those people who gave plus 4 results and those who gave plus 3

results, with a history of definite contact with cases of pulmonary tuberculosis, were examined radiologically at the Civil Hospital, Amritsar. Out of 18 skiagrams, 17 showed tubercular infiltration of the lungs.

Tuberculin survey of school children and young adults, Medical School, Amritsar.

Table 1.

Analysis of the survey work (tuberculin survey) done on the school and college boys.

Total return

Total.	Positives.	Negatives.	Untraceables.	Positive %
2451	1290	994	167	56.5%

Mohammedans

Age Groups.	Total.	Positives.	Untraceables.	Negatives.	Positive %
0-1	1	0	0	1	0.0%
1-5	69	27	1	41	39.7%
5-10	510	245	31	234	51.1%
10-15	415	233	23	159	59.4%
15-20	98	74	5	19	79.6%
20-25	59	46	2	11	80.7%
Total.	1152	625	62	465	57.3%

Hindus & Sikhs.

Age Groups.	Total.	Positives.	Untraceables.	Negatives	Positive %
0-1	1	0	0	1	0.0%
1-5	33	9	6	18	33.3%
5-10	698	301	66	331	47.6%
10-15	159	88	16	55	61.5%
15-20	316	196	12	108	64.4%
20-25	92	71	5	16	81.6%
Total.	1299	665	105	529	55.7%

Table 2.

Table results by communities for the Mantoux test done in ward 9 (house to house) Amritsar.

Total number of families :—462

Hindus & Sikhs

Age Group.	Total.	Positive.	Negative.	Positive %
0-1	5	0	5	0.0%
1-2	8	0	8	0.0%
2-3	11	3	8	27.3%
3-4	12	3	9	25.%
4-5	10	2	8	20%
5-10	36	19	17	52.8%
10-15	24	14	10	58.3%
15-20	10	6	4	60%
20-25	9	9	0	100.0%
Total.	125	56	69	44.8%

Mohammedans.

Age Group.	Total.	Positive.	Negative.	Positive %
0-1	24	0	24	0.0%
1-2	37	4	33	10.8%
2-3	53	9	44	17.0%
3-4	43	15	28	34.9%
4-5	43	15	28	34.9%
5-10	197	88	109	44.7%
10-15	152	90	62	59.2%
15-20	73	49	24	67.1%
20-25	46	35	11	76.1%
Total.	668	305	363	45.7%

INDIGENOUS DRUGS.

34. Indigenous drugs enquiry by Brevet-Colonel R. N. Chopra, I.M.S., at the School of Tropical Medicine, Calcutta.

INDIGENOUS DRUGS.

(1) *Medicine Plants.*—The chemical composition and pharmacological action of *Periploca aphylla* and *Tylophora asthmatica* have been worked out. The glucoside and the alkaloid isolated from the two plants respectively are not physiologically very active. Further work has been carried

out in connection with the alkaloids of *Holarrhena antidysenterica* and *Rauwolfia serpentina*. The pharmacological action of conessine and its isomers iso-conessine and neo-conessine is very similar; their amoebicidal properties are being worked out. Experiments showed that the total alkaloids of *Rauwolfia serpentina* have a more powerful hypnotic action than the individual alkaloids. Electro-physiological studies show that the action of serpentine on the discharge of nerve impulse is more potent than that of the serpentine group in equivalent dilution. Further work on the glucosides obtained from *Antiaris* toxicaria shows that they have powerful digitalis-like effects and in large doses are powerful cardiac poisons. The action of the glucoside isolated from *Cerbera odallum* has been worked out. Its action on the cardiovascular system closely resembles the digitalis glucosides; it is not so powerful but appears to be more prolonged. An essential oil in proportion of about 0.225 per cent. has been isolated from the root of *Hemidesmus indicus*. This is under investigation. The saponin isolated from *Entada scandens* in large doses has a paralytic action on the respiratory centre; it has some action on the striated muscles. The effect of Seitz filtration on the saponin isolated from *Baringtonia acutangula* were studied. The haemolytic activity is lost after filtration.

Other drugs under investigation are :—(a) *Lasiosiphon eriocephalus* contains a glucoside and a phenolic compound and work is being carried on to get these in pure condition. (b) *Solanus trilobatum* contains an alkaloid which is being purified. (c) The chemical composition of *Bambosa arundinacea, Hydrocotyl asiatica* and *Phodomytus tomentosa* is being worked out.

A herbarium of medicinal plants growing in India is being collected with funds given by the Imperial Council of Agricultural Research and the Endowment Fund of the School. It now comprises of about 1500 species or about 5000 specimens but a large amount of work still remains to be done as the list prepared contains more than 2500 medicinal plants growing in India. This herbarium when completed will be a unique collection. It has helped considerably in accelerating the work of this enquiry by giving us means of obtaining authentic specimens of plants for chemical and pharmacological studies.

(2) *Poisonous plants of India.* During the last three years much work has been done in connection with poisonous plants. A large amount of useful information was obtained by sending out 3,000 circulars to various Government Departments, Universities and individual workers all over India which has been analysed. The number of plants poisonous to man, livestock, insects, etc., growing in this country is in the neighbourhood of 700. These are being gradually worked out and preliminary chemical examinations of a number of important ones are being made. All information, botanical, chemical and pharmacological, in connection with them, is being put in book form which is now ready for publication.

(3) *Cobra venom.* Investigations, both biochemical and therapeutic have been carried out on snake venoms which are used in indigenous medicine. Last year a reference was made to the pain relieving properties of cobra venom. Therapeutic trials carried out during the year show that this venom has a definite place in therapeutics in connection with relief of pain of malignant disease, of rheumatic origin and of nerve leprosy, which is the predominant type in this country. As regards the venom

of Daboia, there is some evidence to show that intramuscular injections have some effect in contrplling internal haemorrhage.

A comparative biochemical study of cobra and Russell's viper venoms was carried out. The viper venom contains more of the albumen but less of the pseudo-globulin portion than that of the cobra. Euglobulin is absent in cobra venom but viper venom contains it to the extent of 3.6 per cent. None of the venoms contain any invertase or diastic enzymes, but they both possess the property of digesting fibrin, liquifying gelatin, clotting milk and also digesting casein. The viper venom has a more powerful proteolytic property than cobra venom. An active lecithinase is present in both venoms. Cobra venom is haemolytic in varying degrees for susceptible varieties of red blood corpuscles but viper venom is haemolytic only to a limited extent.

Drug addiction.

As the drug addiction enquiry was closed in 1936, some of the important work which required completion has been carried on by this enquiry with the help from the Endowment Fund of the School. During the year under review investigations on opium smoking habit in India were completed and the results published. It has been shown that while the habit of smoking opium is particularly prevalent in Assam and the Central Provinces it is met with in one form or other on a small scale in many of the large towns in India. The habit has considerably declined during the last 30 years. The method of preparing opium for smoking, the modes of indulgence, the type of persons addicted were studied. In areas where this practice is common, social and economic factors were instrumental in the spread of the habit. The main causes of addiction were association with other addicts, disease or minor ailments, hardwork, worry and strain. The dosage in a series of 300 cases studied ranged from 1 to 180 grains of opium daily. The effects produced by the habit were studied in detail. The conclusions briefly are that excessive indulgence produces, (1) economic loss by reducing the earning capacity of the addicts; (2) damage to health and loss of physical energy and deterioration of intellect; (3) curtailment of longevity.

Work was also continued on the hemp drug habit in India. This is now nearing completion and will be ready for publication in the near future.

Chemotherapeutic and pharmacological investigations.

A simple and fairly accurate method of estimating atebrin in the tissues has been worked out, the more drastic method of dissolving out the tissue materials by means of strong alkali, which might destroy the drug, being replaced by the solution of protein materials with papain.

Work carried out in collaboration with late Col. Acton some years ago showed that dextro-rotatory derivatives of cinchona alkaloids were more potent therapeutic agents than their laevorotatory isomers. A series of these compounds were therefore synthesised in our laboratory and a systematic investigation of their pharmacological action is now in hand. It has been shown that the haemolytic activity of hydrocupreine derivatives increases in potency with the increase in the number of certain atoms in the side chain.

The effect of a number of cardiac drugs was studied on heart explants from chick embryos 2 to 7 days old cultivated *in vitro*. Cardiac nerves

do not develop at this stage and such preparations were employed to determine the exact nature and site of action of lanadagin, thevatin, strophanthin, caffeine, and cardiozol. All these drugs appear to possess a direct action on the cardiac musculature. Lanadagin, thevatin and strophanthin are predominantly inhibitory, while caffeine and cardiozol are stimulant. Caffeine enables the heart explants to continue pulsations for a longer period than the controls. The significance of this is not yet clear.

Experiments were carried out with a view to investigate the effects of varying doses of different antimalarial drugs upon the infectivity of the patient to the mosquito in collaboration with malaria transmission enquiry. The drugs used in these experiments were cinchona febrifuge, quinine sulphate, malarcan, tebetren, atebrin, plasmochin, gametochin and cilional (certuna). All these drugs failed to prevent the development of crescents in *A. stephensi* with the exception of plasmochin and cilional (certuna). Plasmochin in a total dosage of 0.02 gm. could prevent development of the crescents in *A. stephensi* while in case of cilional a total dose of 0.36 gm. was necessary.

35. Enquiry on the study and investigation of certain indigenous drugs of the Punjab by Dr. Khem Singh Grewal at the King Edward Medical College, Lahore.

Rasaut.

1. Investigation on Rasaut is being continued because of the variability in composition of the samples of *Rasaut* as shown by the last year's investigation, a large quantity of *Rasaut* has been reserved for pharmacological investigation. This sample has ash contents of 3.34%, cold water soluble extract of 82.2%, alcohol (90%) soluble extract 31.0% and berberine alkaloid contents of 3.5%. It has traces of other alkaloids oxycanthine and berberine. Toxicity of *Rasaut* has been compared on frog with berberine sulpahte. 0.1 gm. of berberine sulphate per kg. body weight was lethal to all frogs and 0.05 gm. per kg. body weight killed 50 per cent. The lethal dose of *Rasaut* for frog was 1 gm. per kilo and 0.05 gm. per kg. body weight killed 40 per cent. of frogs. Doses of *Rasaut*, which were not lethal within 8 hours, produced extreme irritation of the lymph sac with superficial ulceration of the skin. Berberine sulphate causes only little irritation. Toxicity experiments showed that, in addition to berberine, *Rasaut* contained a substance which was responsible for its toxicity. Attempts have been made to isolate this principle which appears to be a glucoside but it has not been possible to isolate it in a pure state. The effect of *Rasaut* has been compared with berberine on blood pressure and respiration on dogs.

Mamiri.

2. Fresh sample of Mamiri was procured from Solon—The plant was identified with the kind help of Botany Department of the Punjab University as *thalictrum jawanicum*. It contains large quantities of berberine. More of the drug is being obtained for quantitative estimation. It is a curious fact that berberine containing plants are used for infections of the eyes. *Mamiri* and *Mamira* (Coptis Teeta) are very much lauded in the treatment of eye diseases and both have been shown to contain berberine. Except on leishmania tropica, the antiseptic action of

berberine on micro-organs has not been studied. Gupta and Dikshit have shown, at Col. R. N. Chopra's laboratories, that berberine is toxic to leishmania tropica in a concentration as high as 1 : 80,000. The antiseptic effects of berberine and *Rasaut* are being studied both in vivo and vitro.

HING.

3. Ten samples of hing, which were procured from reputed sellers of indigenous drugs, were examined and compared with two samples of Asafoetida procured from well known dispensing chemists. One of the dispensing chemists was found to be selling *hing* for asafoetida (B.P.). The sample of *hing*, which he was selling, was grossly adulterated. It had 26.3% of alcohol soluble resin, 33.07% of ash and the adulterant was chalk. Out of the ten samples of *hing*, only one contained alcohol soluble resin over 50 per cent. and ash within accepted limits. Eight of the samples showed alcohol soluble resin below 30 per cent. and ash contents above 25 per cent. for those adulterated with gypsum and below 10 per cent. for those adulterated with resin.

The result of analysis of ten samples of "Hing" and two samples of asafoetida (B.P.) is as follows :—

Sample No.	Solubility in alcohol.	Ash contents.	Adulterant.
1.	21.034%	11.00 %	Gypsum & Rosin
2.	15.80 %	47.00 %	Gypsum
3.	14.508%	6.250%	Rosin
4.	18.186%	5.07 %	,,
5.	58.520%	9.09 %	Pure
6.	21.924%	35.615%	Chalk
7.	29.97 %	37.04 %	,,
8.	16.53 %	4.45 %	Rosin
9.	43.26 %	25.227%	Chalk
10.	25.29 %	46.1 %	,,
11.	26.332%	33.07 %	,,
12.	76.82 %	3.15 %	Pure

Essential oils of sample Nos. 3, 9, and 12 were prepared, and the refractive indices were determined at 24° C. These were respectively as follows :—

1.5450, 1.5360 and 1.525.

CASSIA TORA, RASNA AND ARUNI.

4. Preliminary work has been done on these drugs Cassia Tora contains emodine principles but others contain no active principles in appreciable quantity. Therefore further investigation is not being undertaken ; the chemistry and pharmacology of Cassia Tora is being investigated.

CENTAURIA BEHEN.

5. The chemical investigation of Centauria behen is being continued Further work is necessary to isolate the active principle.

This investigation has attracted the attention of vaids of Lahore and a committee consisting of the Principal of the Ayurvedic College as convenor and four other distinguished vaids and myself has been formed. Two meetings have been held during November and December, 1938, and it is hoped that the work of this enquiry will be greatly facilitated by their co-operation.

MATERNAL MORTALITY.

36. Enquiry into maternal mortality in Bombay by Miss J. Jhirad.

This enquiry was conducted for a year ending June 30th, 1938. The Executive Health Officer, Bombay Municipality, sent in forms of female deaths regularly and from these the maternal deaths were selected and investigated completely. A certain number of cases returned as not connected with pregnancy, labour or puerperium was also investigated to verify the statement.

The enquiry was conducted by a whole time investigator during the first eight months, who was unfortunately unable to proceed with the work owing to illness, and the work had to be completed with the assistance of two part time workers.

During the year 525 cases were investigated. Of these 10 cases were not strictly within the term "maternal mortality" and were omitted. Of the 515 maternal deaths, 175 were due to associated diseases and 340 were directly due to child-bearing, giving a percentage of 66.1. The classification as per International List (1929) with added headings for anaemia and acute yellow atrophy is as follows :—

140.	Abortion with septic conditions		10
141.	Abortion without septic conditions being mentioned (including haemorrhage)		6
142.	Ectopic gestation		6
143.	Other accidents of pregnancy (haemorrhage excluded) vesicular mole		1
144.	Puerperal haemorrhage		32
	(a) Placenta praevia	11	
	(b) Other haemorrhages	21	
145.	Puerperal septicaemia (not specified as consequent upon abortion)		133
	(a) Puerperal septicaemia or pyaemia		
	(b) Puerperal tetanus		
146.	Puerperal albuminuria and eclampsia		36
147.	Other toxaemias of pregnancy		6

148.	(Puerperal phlegmasia alba dolens) embolism or sudden death (not specified as septic)	21
149.	Other accidents of child-birth—difficult labour	18
150.	Other or not specified conditions of the puerperal state—	
	Puerperal insanity	3
	Anaemias	58
	Acute yellow atrophy	10
		340

It will be noticed that sepsis accounts for more than one-third of the total deaths from child-bearing. 43.60% of these were in the practice of dais. Anaemia stands second in order of frequency. Eclampsia including albuminuria, and puerperal haemorrhages run almost parallel.

The year under enquiry was marked by two general epidemics of smallpox and enteric fever which helped to swell the figures under associated diseases, and yet in spite of this, tuberculosis accounts for the largest number of deaths due to associated diseases.

MATERNAL MORTALITY RATE.

The total births registered during the period under investigation were 38,243 (including 2451 still births) and the total maternal mortality 340, giving 8.88 per 1,000 births. Two hundred and thirty-four cases or 68.53% showed an avoidable factor. It was noticed that only 24.1% received ante-natal care. A full report is under preparation.

37. Anaemia and eclampsia enquiry in Calcutta by Dr. L. E. Napier.

This enquiry has suffered from changes both in the personnel and directorate during the year; Dr. Neal Edwards went on leave early in the year and Dr. L. E. Napier took charge of the enquiry; the latter was on leave for 2 months during which time Dr. Orkney kindly looked after the enquiry. Dr. Pundit left early in the year, and shortly afterwards Dr. Dharamvir left. Finally, Dr. Mukerjee left at the end of September. On each occasion the vacant post has been filled without much delay and quite satisfactorily, but there has naturally been some breaking of the continuity of the work.

The scheme laid down by Dr. Neal Edwards has been followed. Cases of anaemia in pregnancy have been examined and followed. Most of the cases were originally seen in one of the large hospitals, at maternity and child welfare clinics, or elsewhere, but have been followed to their homes where the examinations were continued, usually until their blood picture returned to within the normal limits. Up to the present 427 such cases have been examined, and 622 home visits paid.

The opportunity arose for examining the bloods of normal pregnant women; 56 such women were examined. Complete blood examinations were also done on 135 normal non-pregnant girls and women. The results when analysed will provide valuable data for future work.

38. Enquiry into anaemia of women by Dr. L. E. Napier at the School of Tropical Medicine, Calcutta.

Staff and location.

During the whole of the year Dr. Majumdar has remained in Calcutta. His time has been almost fully occupied in doing blood counts on a number of monkeys which have been under experiment, but he has also assisted with the investigations on anaemia in patients in the hospital.

In connection with this enquiry, a diet survey was carried out in Dr. Manson's practice at Jorehaut in Assam, in March and April, by Dr. D. Mitra, the diet survey officer working under Dr. Wilson, and now under Dr. Sankaran, at the All-India Institute. Dr. Napier also visited Jorehaut at this time to collect certain data and to carry out some haematological examinations in connection with this diet survey and his previous investigation in this district.

Diet surveys.

During the year the results of the diet survey carried out by Dr. Mitra at Dewan in connection with the investigations of this enquiry were published. The data he obtained at Jorehaut are being analysed.

Investigations on pregnant anaemic coolie women at Dewan.

During the year the case cards of this investigation, which was completed last year, were analysed and a paper on the subject was published in october 1938 issue of the I.J.M.R.

This paper can be summarized as follows :—

An examination of the blood of 25 males and 25 females showed a low standard of haemoglobin amongst the so-called normal coolies in the population from which the cases were taken. The mean corpuscular volume and the mean corpuscular haemoglobin were also below normal. This is the common characteristic of coolie populations ; the significance is discussed.

The cases investigated were provisionally divided into three groups as follows :—

A. *Hypochromic.*—There are in this group 26 cases : of these 7 are microcytic, 18 are normocytic and 1 macrocytic ; of these 26 cases, (1) 16 (6 microcytic, 9 normocytic, 1 macrocytic) only received iron and responded to the treatment in varying degrees, (2) 5 (1 microcytic) had iron only and did not respond, (3) 4 had mixed treatment (all normocytic) and responded to treatment in varying degrees, and (4) 1 (normocytic) had mixed treatment and died.

B. *Orthochromic.*—This group consisted of 28 cases, (1) of these 18 (4 macrocytic and 14 normocytic) responded to treatment in varying degrees, (2) 7 died (6 normocytic and 1 macrocytic), and (3) 2 (both normocytic) did not respond to mixed treatment, and 1 received no treatment.

C. *Hyperchromic.*—The three hyperchromic cases were all macrocytic, and all responded to treatment with liver extract.

The response to treatment in these cases is discussed in detail and in 34 cases this is demonstrated by graphs. According to their response to treatment the cases are arranged into two groups, the iron-responding group and the marmite-liver-responding group which correspond very

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closely to the hypochromic group, and the orthochromic and hyperchromic groups, respectively, certain ætiological, clinical and haematological features in the two groups are contrasted, and the special characteristics of each group summarized.

The aetiological factors in these two types of anaemia are discussed, and the important findings in a dietary survey that was carried out in conjunction with this enquiry are given. The following conclusions were arrived at :—

The population of pregnant tea-garden coolie women as a whole has a low level of haemoglobin associated with a small pale red blood cell : conditioned iron deficiency, brought about by continuous blood loss from hookworm infection in the presence of a low iron intake, will account for this in part, but not, it is believed, altogether, and it is considered possible that a low calcium and / or a low vitamin-C intake may be associated factors.

The anaemia amongst pregnant women can be divided into two groups, characteristically microcytic hypochromic and macrocytic hyperchromic, respectively ; the former is an exaggeration of the anæmia in the normal population brought about by the extra demands of pregnancy, but the factors concerned in the latter are not quite so evident, though the response to treatment suggests—and a dietary survey does not negative the suggestion—that there is a relative deficiency in important food factors probably associated with the vitamin-B_2 complex, and other observations indicate that this deficiency is conditioned by the presence of the foetus with its extra demands for substances essential for haemopoiesis (or less probably its toxic influence) and/or by a state of chronic malaria.

Whilst characteristic examples of each type of anaemia are seen, they seldom occur in 'pure' state and in the vast majority of cases deficiencies of both groups are evident and require rectifying.

Experiments with monkeys.

The object of these experiments was to study, under controlled conditions, that would be possible with animals, the anaemia produced by repeated blood loss—the counterpart of hookworm anaemia, nutritional deficiency, and malaria, severally and in different combinations, and later to study the effect of treatment in these anaemias.

It was first necessary to establish normals for comparison, and particularly to watch the effect of captivity on the blood picture in monkeys.

The 11 monkeys used in these experiments and 12 other monkeys were examined and the mean of their normal counts is shown below. In the same table the counts on a control monkey, which showed an average picture, are given ; it will be seen that little change occurred in 9 months of captivity.

Date.	Haemo-globin.	Red cells mills.	Reticu-locytes.	MCV.	MCH.	MCHC.	Weight lbs.
Mean	13.21	6.026	0.65	74	22,1	29.7	-
Control, 24.1.38 . .	13.61	5.980	0.8	77	22.8	29.7	6½
„ 19.10.38 . .	14.30	6,170	1,4	81	23.2	28.6	10

These experiments are still being carried on and no definite conclusions have been arrived at. However, the typical macrocytic picture that Dr. Lucy Wills reports when monkeys are fed on a faulty diet has so far not been produced. The same diet that she advocated has been used, and, though it has been possible to study the effects of repeated malarial infections in monkeys on an ordinary diet, the malarial monkeys fed on a 'Lucy-Wills' diet have unluckily always died.

Since January, Dr. Majumdar has done in all 359 complete full blood counts on these experimental monkeys.

FURTHER NORMAL STANDARDS FROM WOMEN AND CHILDREN IN CALCUTTA.

In conjunction with the anaemia and eclampsia enquiry, Dr. Majumdar has assisted in carrying out blood counts on 'normal' women and children of different classes, as follows :—

Full blood counts on women, aged 15 to 30	130 persons
Haemoglobin and physical data in school-boys	800
,, ,, in school-girls	250

The results are awaiting analysis.

39. Enquiry into toxaemias of pregnancy by Dr. N. A. Purandare at the Seth G. S. Medical College, Bombay.

The preliminary arrangements for the work were commenced from the middle of May 1938 ; but it took almost a month and a half to begin the enquiry as permission had to be obtained from the heads of the hospitals to use the material available in their institutions. The work was therefore, strictly speaking, done in the last five months.

The late toxaemia of pregnancy has been chosen for investigation. Eclampsia can at once be recognised by its most distinguishing feature but to make out cases of preeclampsia, careful examination had to be carried out. It has been established that careful watch over antenatal cases and detection of preeclampsia in the early stage may avert the development of eclampsia, a formidable complication of pregnancy. Preeclampsia sets in in most of the cases insidiously, which entails close attention especially on the primigravida from the seventh month. It is regrettable that the people have not yet come to understand the significance of antenatal supervision and some of the cases come under observation when the toxaemic condition has already been well established. Nevertheless the following methods were instituted to determine the preeclampsia.

(1) GENERAL EXAMINATION OF THE PATIENT INCLUDING SYSTEMIC EXAMINATION.

(2) EXAMINATION OF URINE :—its quantity, appearance, Sp. gr., general analysis, quantitative for albumin, sugar, urea, uric acid, chlorides, phosphates, oxalates.

(3) BLOOD EXAMINATION :—Quantitative ; urea, uric acid, non-protein nitrogen, calcium.

In all 50 blood samples were examined.

(a) *Urea nitrogen* was determined by Follin and Wu method, in 37 cases.

(1) Urea nitrogen was increased in 2 cases.

(2) Urea nitrogen was normal in 17 cases. (9 to 14 mgm. per 100 c.c.).

(3) Urea nitrogen was below normal in 18 cases.

(b) *Non-protein nitrogen* :—41 samples were examined.

(1) Increased in 15 cases.
(2) Normal (20 to 35 mg %) in 34 cases.
(3) Below normal .. 1 case.

(c) *Uric acid* :—46 samples were examined :

(1) Increased in .. .. 31 cases.
(2) Normal (2 to 3 mg %) .. 13 ,,
(3) Below normal in .. 2 ,,

(4) BIOLOGICAL EXAMINATION OF URINE :—Proportion of oestrin and of anterior pituitary like hormone was determined by injecting urine in mice.

(*a*) *Oestrogenic substance*:—It was determined in 4 cases on ovariectomized mice. Tech : Allen & Doisy.

Inference :— Oestrogenic substance varied from 6,500 m.u. to 20,000 m.u. per liter of urine according to severity of the case, oestrin being less in a more toxaemic case.

(*b*) *A. P. L. Hormone*:—It was determined in 7 cases on immature mice.

Tech : Aschheim-Zondek.

Inference :— A. P. L. hormone varied from 500 m.u. to 2,500 m.u. per liter of urine.

In this half year's enquiry, 17 cases could be obtained in the following hospitals :—

	No. of cases.
Wadia hospital	10
Cama ,, ...	4
Motlabai ,, ...	2
Private ,, ...	1
	17

Caste incidence :—

Hindus	8
Mahomedans	2
Christians	6
Jews	1

Of these, two were vegetarians and the rest non-vegetarians.

Age incidence :—

Years	No. of case.
15-20	5
21-25	5
26-30	6
31-35	1

Of these seventeen cases, 6 were primigravidae, 6 second paras and the rest multiparas. Excluding six primiparas, in the remaining 11 cases, there was a distinct history of recurrence of oedema in five, which had lasted from 2 to 3 months. Of the remaining six, there was no oedema in previous pregnancies, in three, while in the rest there is no mention of it at all.

In 11 multiparas, abortions in previous pregnancies had happened in two cases; there were five still-births while one child died within a day birth.

As to severity of cases, four were mild cases, eleven severe and two grave.

Most of the patients were more or less pale in appearance and their condition fair.

SYMPTOMS: the essential characteristic symptoms were oedema, albumin and blood pressure.

Oedema.

(I) At the time of admission :—

(a) absent in 3 cases.
(b) + in 6 ,,
(c) + + in 2 ,,
(d) + + + in 6 ,,

(II) During stay in the hospital :—

(a) increased in 3 cases.
(b) decreased in 9 ,,
(c) stationary in 2 ,,

Of these 17 cases, six have not delivered.

(III) At delivery :—

(a) no oedema .. .. 3 cases.
(b) increased .. .. 1 case.
(c) decreased .. .. 4 cases.
(d) stationary .. .. 3 ,,

(IV) After delivery oedema :—

(a) disappeared in .. 5 cases.
(b) lessened .. .. 3 ,,
(c) no oedema at delivery .. 3 ,,

Albumin

(I) At the time of admission :—

Nil 2 cases.
Slight + 9 ,,
Moderate + + 3 ,,
Excess + + + 2 ,,
Becoming solid on heating + + + + 1 case.

(II) During stay :—

(a) increased .. .. 3 cases.
(b) decreased .. .. 5 ,,
(c) stationary .. .. 4 ,,
(d) disappeared .. .. 3 ,,

(III) After delivery :—

(a) disappeared .. .. 6 cases.
(b) diminished .. .. 2 ,,

Blood pressure :

(I) At the time of admission :—

(a) under .. 130 .. 4 cases
(b) between .. 130 - 160 11 ,,
(c) ,, .. 161 - 180 2 ,,

(II) During stay :—

(a) increased .. .. 7 cases.
(b) decreased .. .. 3 ,,
(but remaing above 130°.)
(c) stationary .. .. 3 ,,
(d) below 130 .. .. 4 ,,

(III) After delivery :—

(a) between 90 to 110 .. 6 cases.
(b) ,, 110 to 130 .. 3 ,,
(c) ,, 130 to 150 .. 2 ,,

TREATMENT : Treatment with :—

(a) Proluton .. .. 5 cases.
(b) Proluton followed by progynon .. .. 3 ,,
(c) Progynon (B) .. 6 ,,
(d) Progynon (B) with calcium gluconate .. 2 ,,
(e) Calcium gluconate injection alone .. .. 1 ,,

Results of treatment :—

(a) Proluton :
(1) no improvement .. .. 2 cases.
(2) increase in symptoms .. 1 case.
(3) doubtful .. .. .. 2 cases.
(In one case foetus died and was expelled macerated,

(b) Prolution followed by progynon (B)
(1) improvement .. .. 1 case
(2) doubtful (as the patients were confined soon after starting with progynon) 2 cases.

(c) Progynon (B) 10,000 & 50,000
(1) improvement .. .. 2 cases
(2) no improvement .. .. 2 ,,
(3) doubtful (as the patients could not be watched long, as they presently delivered) .. .. 2 ,,

(d) Progynon with calcium gluconate.
(1) improvement .. .. Nil
(2) doubtful .. .. ..
(under observation)

(e) Calcium gluconate injections 10 c.c.
(1) symptoms increased .. .. 1 case.

40. Investigation of the biochemical and dietetic factors concerned in the toxaemia of pregnancy by Dr. Vishwanath at the K. E. Medical College, Lahore.

Twenty-one cases of toxaemia of pregnancy were investigated. The biochemical investigations of the blood and urine of these cases gave the following results. Seven of these cases were clinically eclamptic. The diets of these cases were very carefully studied and also of 25 controls of the class attending the free wards of the local hospitals. It seemed that protein and albumin intake in both groups was low.

1. Hypoproteinaemia existed in all the cases studied. It showed greater diminution however in eclamptics than in cases of nephritic toxaemia.

Limits :—Total proteins in the blood 4.3% to 5.5% eclampsia.
5% to 5.2% nephritic toxaemia.
5.8% normal pregnant controls.

2. Blood cholesterol was higher in the toxaemic cases than in the normal pregnant controls. Its level in the latter was lower than is the case in Western countries.

Limits :— Blood cholesterol. 176 mgs.% to 250 mgs.% in toxaemic cases.
150 mgs.% to 200 mgs.% in normal pregnant controls.

3. Haemoglobin was consistently low in all toxaemic cases, as compared with the controls.

Limits :—Haemoglobin. 50% to 80% in toxaemic cases.
70% to 90% in normal pregnant controls.

4. There was no significant alteration of blood sugar in toxaemic cases or normal controls.

Limits ;—Blood sugar. 106 mgs.% to 128 mgs.% in toxaemic cases.
100 mgs.% to 130 mgs.% in normal pregnant controls.
210 mgs.% in one case of diabetic coma.

5. Subnormal urea clearance with oliguria was present in cases of eclampsia, only during the acute phases of the disease.

Limits :—Urea clearance. 63% to 41%.

6. Blood calcium which stood at 9.2 mgs.% in the controls was found to be only 8.03 mgs.% in the eclamptics.

Limits :—Blood calcium. 7.6 mgs.% to 9 mgs.% in eclamptics.
8.8 mgs.% to 9.6 mgs.% in normal pregnant controls.

7. The urine of all the cases was tested by the Tilman method for the determination of vitamin C content. Only five cases showed normal excretion and they belonged to the control group. The eclamptic cases showed no excretion of vitamin C in their urine.

41. Enquiry into the haematological standards of healthy Indian women by Alice Benjamin at the Lady Hardinge Medical College, New Delhi.

The need for definite haematological standards for Indian women has often been emphasized. The object of this investigation was to compare and confirm the standards obtained by other workers in India.

The subjects of examination were a hundred healthy females, residents of Delhi, between the ages of 17 and 30. They were non-pregnant and no account was taken of their diet or menstrual cycle.

Methods.

All tests were made on venous blood. 5 c.c. of blood were drawn from the median cubital vein without causing undue congestion of the limb and thoroughly mixed with 10 mg. of neutral potassium exalate. *Red cell count* :—From two to four closely agreeing counts were made on each sample of blood, the duplicate counts varying by less than 200,000 cells per c.m. Levy-Hansser counting chambers and Trenner pipettes both tested by the American Bureau of Standards, were used.

Haemoglobin :—The haemoglobin was estimated by the oxygen capacity method of van Slyke & Neill, working on the assumption that 1g. of haemoglobin combines with 1.34 c.c. of oxygen. At least two estimations were made and the mean of two closely agreeing results was taken. As the Hellige method is in use in a large number of Hospitals, the van Slyke not being convenient for clinical work, the haemoglobin of each sample was also estimated by the Newcommer and Hellige instruments. Both are calorimetric methods where the haemoglobin is converted to acid haematin and compared with colour standards. The latter is standardised so that 100% represents blood containing 17 g. oxyhaemoglobin in 100 c.c.

Cell volume :—Wintrobes haematocrit tubes were used. One c.c. of the oxalated blood was centrifuged at 3500 r.p.m. for half an hour, after which time it was found that no further packing occurred. The cell volume thus obtained was corrected for the shrinkage caused by the potassium oxalate, by multiplying the result by 1.09. The values stated in this paper are uncorrected.

Red cell counts.

The average for a hundred women was 4,560,000 per c.mm. the values ranging from 3.99 to 5.44 millions per c.mm.

Mean = 4,560,000 $\pm$ 0.017 million.
Median = 4,510,000 $\pm$ 0.022 ,,
The standard deviation = 0.255 $\pm$ 0.012
The coefficient of variation = 5.59 $\pm$ 0.268

The significant variation was 4.32—4.82 and 74% of the subjects fell within this range.

Haemoglobin content.

The results of the estimation by the three different methods showed a fairly close correspondence. The averages were as follows :—

Van Slyke	= 13.11 g. per 100 c.c.
Newcommer	= 13.02 g. per 100 c.c.
Hellige	= 12.83 g. per 100 c.c.

With the Van Slyke method the average for a hundred women was 13.11 g. per 100 c.c. of blood corresponding to an oxygen capacity of 17.6 c.c. The values ranged from 11.14 to 15.00 g.

The mean = 13.11 ± 0.055
The median = 13.16 ± 0.069.
The standard deviation = 0.814 ± 0.039.
The coefficient of variation = 6.209 ± 0.298.

The significant variation was 12.30 — 13.92 and 70% of the cases fell within this range. The average mean corpuscular haemoglobin (M.C.H.) worked out at 28.73 rr (micromicrograms), the values ranging from 24.88 rr to 32.03 rr.

Cell volume.

The average was 39.04 c.c. per 100 c.c. of blood, the values ranging from 33 c.c. to 44 c.c.

The mean = 39.04 ± 0.164.
The median = 40.19 ± 0.207.
The standard deviation = 2.448 ± 0.118.
The coefficient of variation = 6.273 ± 0.301.

The significant variation was 36.69 — 41.49 and covers 66% of the cases. The average mean corpuscular volume worked out at 93.2 c.m. (cubic microns).

Colour index.

The average colour index for 100 cases was 1.00, the values ranged from 0.85 to 1.11.

Table I.

Observer.		No. of cases.	Red cell count.	Haemoglobin.	Cell volume.	
Osgood & Haskin ..	1927	100	4 80	13.69	41.04	European.
Sachs Levine & Fabian ..	1935	100	4.51	12.96	..	
Wintrobe	1930	50	4.93	13.76	39,5	
Price Jones	1931	100	5.01	13.50	..	
Haden	1912	12	4.26	12.93	..	
	1933	30	4.38	13.37	..	
Sokhey	1929	101	4.47	12.99	36.27	Indian.
Sankaran & Rajagopal ..	1938	62	..	13.73	..	
Values observed in this investigation	1938	100	4.56	13.11	39.04	

In Table I are collected the estimations made by several other observers. The values observed in this investigation for the red cell and haemoglobin agree very closely with those of Sokhey in 1929, and with some of the European figures. The average cell is slightly higher than the figure obtained by Sokhey. It has often been stated that the haemoglobin of the Indian is lower than that of the European ; there appears no evidence in support of the statement as the two sets of figures are fairly close.

42. Preliminary survey for the investigation of the bacteriology of the genital tract by Lt.-Col. S. N. Hayes, I.M.S. at the Lady Willingdon Hospital, Lahore.

The scope of the enquiry was restricted in the year 1938-39 "To investigate the bacteriology of the genital tract during pregnancy and the puerperium in all emergency cases admitted to hospital. The investigation to be particularly concerned with the identification of streptococci".

In addition, it was proposed to describe the technical difficulties encountered owing to the climatic conditions of the Punjab.

The following work was also carried out :—

(1) Throat swabbing and isolation of Beta haemolytic streptococci from cases whose genital tracts were swabbed for investigation.
(2) Throat swabbing of the staff and the students.
(3) Blood culturing of the cases of post-partum pyrexia.
(4) Investigation of methods of isolation of anaerobic streptococci.

The following groups of cases were examined up to the 11th November, when the enquiry was closed down by instruction of the Secretary, I.R.F.A. :—

	Antenatal.	During labour.	Postnatal.
Vagina and cervix.	113	207	107
Throat.	101	151	47

Disparity in figures of swabbing throat and genital tract in each group of cases is due to the fact that throat swabs could not be taken in some of these cases.

Percentages of cases positive for Beta haemolytic streptococci, in the above groups, were as follows :—

	Antenatal.	During labour.	Postnatal.
Vagina and cervix.	1.76	2.89	37.38
Throat.	5.0	4.63	4.25

Examination of blood cultures :—Blood was cultured aerobically and anaerobically in 67 cases of post-partum pyrexia. Bacteriaemia could be definitely demonstrated in 7 cases only, out of which 6 showed Beta haemolytic streptococci. The seventh (Reg. No. 1022) was infected with a gas-forming gram positive bacillus of the anaerobic group. One of the cases infected (Reg. No. 1337) with streptococci was also associated with a gram negative gas-forming bacillus. This case was not investigated further. Gram-negative bacilli were met with in two other cases (Reg. Nos. 644 and 1192). Investigation of one of these strains (Reg. No. 1192) at the C.R.I. Kasauli, showed that the organism did not exhibit reactions characteristic of the known pathogens. Diphtheroids have also been met with once (Reg. No. 1000). No pathogenic significance, however, could be attached to them.

All the strains isolated up to 31st October, fifty-eight in number, from the genital tract, throat and blood were sent to the C.R.I., Kasauli, for typing. Details will be communicated in a separate and detailed report.

Anaerobic incubation for the isolation of streptococci from the genital tract could only be carried out in a small number of cases, as the anaerobic jar available was small. Anaerobic streptococci could not be isolated from the swabs or blood. Further work, in this connection, is needed as the clinical evidence points to the frequent occurrence of infection by these organisms.

A preliminary attempt was made to study the epidemiological aspect of the problem by swabbing the throats of the staff and the students. It was found that 7.14% were harbouring Beta haemolytic streptococci.

Investigation of the association of the carrier showed that 54 per cent. of abnormal labour cases conducted by him were pyrexial in the puerperium.

Further identifications to prove the similarity of the infecting orgnisms were not possible.

Epidemiological investigations, beyond the grouping of the strains isolated (at the C.R.I., Kasauli) were not taken up further as it was not within the scope of this enquiry.

MISCELLANEOUS.

43. Helminthological enquiry under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta.

The principal work of the year has been the collection of intestinal worms from 100 stray dogs captured in Calcutta. This was completed a short time ago and the identification of the worms has begun. The main object of this investigation is to find out to what extent, if any, *Taenia ovis* is present in dogs in India. This is in pursuance of the idea that at least some of the cases of human cysticercosis in this country are caused by the cysticercus of this worm and not by *Cysticercus cellulosae*. With the object of widening the enquiry to include districts where a considerable number of cysticercus infections in British troops appear to have been acquired arrangements were made to obtain dog tape worms from the Punjab and the North West Frontier Province, but the undertakings to send parasites from these places have not been fulfilled. The budget of this enquiry does not permit of a special tour all the way from Calcutta to these and other districts to collect worms from dogs, so the enquiry if it proves wholly negative for Calcutta, will not give conclusive evidence for the rest of India, because it is quite likely that the incidence of intestinal parasites in dogs varies in different parts of the country in the same way that parasites of human beings and other animals are known to do. Therefore to make this enquiry complete material is urgently needed from areas other than Calcutta.

Another research that has been occupying our attention for a long time is the question of preserving hookworm eggs in stools, because when

a stool has to be kept for days and in some cases even weeks, and perhaps transported for long distances before they are examined the loss of eggs is very great, so that a survey based on material of this nature may give a false sense of security regarding the severity of infection in a given district. Some years ago after a long series of experiments freshly prepared antiformin was found to be fairly reliable, but the objection to it is that it is an unstable compound and unless carefully prepared and stored the chlorine content is liable to fall so low that it looses its effect. It has therefore always been felt that a more stable compound should be found, if possible. In the intervening time very many substances have been tried without success until this year, when we have had very promising results with a quite different substance. As several others have appeared good for a time but on further trial have proved ineffective in some instances, the results with the new substance are not going to be published until they have been confirmed by trial throughout at least one more hot season.

Attempts to improve upon the far from perfect anthelminthic treatments available are continually in progress, and this year a paper has been published giving the results of five years' work with different doses of oil of chenopodium and santonin in the treatment of ascaris infection. Another branch of this work is putting to the test, under local conditions, treatments claimed to be successful elsewhere, and at present we are investigating the claim that large doses of iron are curative of trichuris infection.

The life history of *gnathostoma spinigerum* has been found to follow the same lines as in Siam. The original failure to infect fish here was probably because all species are not susceptible and in the first instance an unfavourable host was selected. Specific susceptibility of fish to this infection has not been demonstrated either in Siam or the Phillipine Islands where all kinds of fish used have become infected. This immunity of certain fish in Bengal may explain the rarity of the infection in human beings.

44. Medical mycology enquiry under Dr. P. A. Maplestone at the Calcutta School of Tropical Medicine.

Work on testing of fungicides has been continued. The paper published at the end of the last financial year was by no means complete, and this work has been continued with the object of filling the gaps, and adding to the facts already elicited. Altogether 18 substances have been used in these experiments and in the first series of results published, eight were tried on four widely different species of fungus and the other 10 were only tested against 2 of them. The results with the four different fungi were very different with most of the materials tested, and as this research is concerned with finding the best possible fungicide for general purposes, the remaining 10 substances are now being tested against the other two fungi as well. Also in the preliminary investigations the different strengths of the various substances tried were fairly widely separated so that the difference between the highest effective dilution and the nearest non-effective one is fairly large. Work is now being done with dilutions between these two so as to get a more accurate knowledge of the highest dilution that will kill these fungi.

There was an outbreak of microsporon infection in the hair of children in a boarding school in Shillong last year and seven children were affected. Some of these children were sent to Calcutta and Major Haythornthwaite kindly collected material from the others. The seven samples of material were cultured separately and they were found to be all the same species of fungus, but different from another microsporon recovered from a case in another Shillong school about the same time. The latter proved to be *M.audouini* which is the common cause of ringworm of the hair in India. The species recovered from the seven children was found to resemble *M.ferrugineum* which was first described by Ota in Japan in 1922 and since then in other eastern countries, but never in India until the present time. A type culture of *M.ferrugineum* has been obtained from Prof. Ota and the Shillong species appears to be a variant from the type. A paper on this fungus is now in the press.

The study of the aetiology of cheiropompholyx of the hands to decide its connexion with ringworm of the feet has led to the investigation of 42 cases. It is still not settled how many are local infections of the hands, how many are dermatophytides caused by toxins arising from infection on the feet, and how many cannot be ascribed to either of these causes. The subject has been approached in three ways. The feet were carefully examined for evidence of ringworm infection, the blisters of the hands were examined in the same way, and evidence of skin sensitization is tested for by the use of a trichophytin (fungus extract) prepared by a special technique from cultures of *Epidermophyton floccosum* taken from the toes, fingers and nails of local cases. A similar substance prepared from several different types of fungus in South America has been used on the same cases, and no differences have been noted in the reaction produced with the two preparations. From this it appears that the cutaneous reaction is more of a group reaction than one confined to individual species of fungus. In the light of this observation the scope of this work is being extended by preparing extracts from botanically widely differing fungi such as epidermophyton, microsporon and monilia and testing them separately on the same cases.

The American preparation was originally sent with a request to try it as a curative agent in resistant cases of ringworm as it was claimed to be successful in South America. After extensive trial this claim has not been confirmed; so it is with the idea of finding out if the local failure is to be explained by specific differences in the fungi of the two countries that our present line of work is being followed.

In the study of the supposed virus diseases of the skin the clinical results continue to be successful, but isolation of the specific viruses by inoculation on the chorio-allantoic membrane of eggs appears to be difficult if not impossible in the majority of them. Although a considerable degree of inflammatory reaction usually occurs, isolated specific lesions or pocks as they are called do not appear. Dr. Burnet, a noted authority on virus work, has suggested to me in correspondence that these viruses are probably among the ones that will not grow on eggs. In view of this, work has been carried on this year to see if there is specific antibody formation in cured cases. This has necessitated the preparation of a specific antigen for each disease.

The three diseases so far dealt with in this way are herpes, molluscum contagiosum and infective warts and the first method of antigen preparation was as follows and was the same in all three diseases. Saline and

alcoholic extracts were made from the membranes of eggs showing infection, and also from the liver and heart of chick embryos of the same eggs. The saline extracts were found to be free from anti-complementary and haemolytic action so they were employed in testing the sera of recovered cases of these three diseases. Without going into detail it may be said that the results are not very good as only about 2 per cent. of just over 100 cases, taking all three diseases together, gave a positive reaction. The alcoholic extracts were anti-complementary and so were not used.

As the antigen produced in the above manner does not seem very powerful an attempt is being made to enrich it by the addition of cholesterol, and another altogether different method of antigen manufacture is also being followed. This is to use the brains of rabbits that have been inoculated with material from the skin and which subsequently show signs of infection. This has as yet only been done with molluscum contagiosum, and an alcoholic extract of dried brain has been found to be not anti-complementary and not haemolytic. Only one suitable case has as yet become available and it gave a very definite complement fixation reaction. So this line of enquiry appears worth following up.

Although complement fixation in lung tuberculosis has been extensively studied, as far as we are aware, this has not been done in tuberculosis of the skin. Up to the present 11 cases of lupus vulgaris have been studied from this point of view using the Witebsky, Kingestein and Khun antigen. Lupus vulgaris and not tuberculides were chosen because the former have been definitely proved to be caused by the local action of the tubercle bacillus while there is still doubt as to the true cause of tuberculides. These will be studied later, and if found positive will give valuable support to the idea that they are of tuberculous origin. Seven of the 11 cases were negative and one gave a doubtful reaction. Of the other three which were definitely positive two had a history of recent haemoptysis and the other had longstanding chronic lung disease.

45. Dracontiasis & filariasis enquiry by Dr. S. Sundar Rao at the Calcutta School of Tropical Medicine.

A. Dracontiasis.

During the period under review some field-work was carried out on dracontiasis in several endemic areas namely the districts of Vizianagram, Trichinopoly, Ramnad and Madura. At the field station located at Vizianagram, it was observed that the incidence of cyclops in wells and ponds was high during the dry months and that, with the onset of rainy season, the number of cyclops decreased progressively. This finding is of interest in view of the fact that guinea-worm infection is most frequent during the dry season.

For the preparation of an antigen for complement-fixation test in guinea-worm infection, adult guinea-worms were collected. In collaboration with Major Greval an antigen was prepared from this material by a special technique. Blood samples collected from cases in the endemic areas were tested for complement-fixation. In all cases so far tested, where the host actually harboured worms, the tests gave uniformly positive results. In persons without any active worms in the body as well as those in whom the worm had been removed by extraction, the tests gave negative results. These observations are of interest in view of the fact that Hamilton Fairley

and Liston did not obtain any definite evidence of complement-fixation with the guinea-worm antigen in the cases that they investigated.

Complement-fixation tests with the same guinea-worm antigen were carried out in *Wuchereria bancrofti* carriers and also in cases of elephantiasis. The results of either series of tests were negative. From the present experience it is unlikely that this antigen could be employed for detecting filarial infection in a subject by complement-fixation.

Specimens of cyclops collected from endemic as well as non-endemic areas were identified. No apparent difference was observed in the cyclops fauna of the two areas. Species of cyclops are not factors in the transmission of this infection.

Through the co-operation of medical officers of different parts of India the School now possesses a large collection of adult *Dracunculus medinensis*.

B. Filariasis.

In regard to filariasis, field investigation and laboratory work were carried out. A second filariasis survey was made in Patnagarh (Orissa), a locality with a high incidence of *Microfilaria malayi* infection. Attempts were made to obtain the adult of *Microfilaria malavi* by removing enlarged glands of carriers and examining them for adult worms. So far the attempts have not been successful.

Sternal punctures were carried out in *Wuchereria bancrofti* carriers. It was observed that microfilariae occurred in the bone marrow only during night and were absent from it during day time. The number of microfilariae in the bone marrow was very small as compared with those in peripheral blood and in venous blood.

Cholestrol and lecithin content of blood were estimated in normal persons and in persons showing *Microfilaria bancrofti*. It was observed that the cholerstrol content was slightly higher in the carriers than in normal persons. The cholestrol content of hydrocele fluid and of lymph from elephantoid cases is now being investigated.

Generally the onset of chyluria is the result of rupture of varicose lymphatics in the bladder wall. The usual treatment of this condition is the administration of antiparasitic drugs or calcium and washing the bladder with astringents to heal the lymphatic rupture. Pectine, which is a well-known coagulant, has been employed with remarkable success in such conditions. In all the cases treated with Pectine the urine became clear in 2 to 3 days.

Several new drugs were tried on cases with *Wuchereria bancrofti* infection namely : cuprion, surfen, cilional and sdt-561 of Bayer. Synthalin B, May & Baker 693 and Ext. rawalfia liq. None of them had any lethal effect on the parasite.

46. Protozoal parasites enquiry under the Director, King Institute, Guindy, Madras.

This enquiry was under the direction of Lieut.-Colonel H. E. Shortt, I.M.S., Director, King Institute, Guindy, until 4th October 1938, when it was transferred to Coonoor under Major H. W. Mulligan, I.M.S.

The work of the enquiry during the year was directed mainly towards the collection and preparation of material, most of which has not yet been subjected to detailed study. Much of the time of the personnel was occupied in the laborious tasks of establishing strains, feeding insects and subsequently preparing the latter for histological examination. Most of this material has been taken to the London School of Tropical Medicine and Hygiene, where it will be examined by Col. Shortt. Such preliminary examinations as have been made indicate that the material collected promises to produce interesting and important results. A brief outline of the work carried out during the year is given below.

(1) DEVELOPMENT OF *Babesia gibsoni* IN *Haemaphysalis hispinosa.*

Nymphs and larvae of this tick were infected with *Babesia gibsoni.* Successful transmission of the parasite was obtained in only six cases out of a very large number of transmission experiments. This suggests that the development of the parasite occurs in only a very small proportion of the ticks fed on infected dogs. A large number of ticks which had fed on infected animals was prepared for microscopical examination. In preliminary examinations of some of this material two ticks showed appearances which were suggestive of the developmental cycle of *Babesia.*

The strain of *B.gibsoni* in use was not very virulent for local dogs. The initial attack may be severe but spontaneous recovery takes place in the majority of cases and the animals show no symptoms subsequently, although parasites may persist in the blood for many months. This was true whether the infection was passaged by tick bite or by blood inoculation.

Attempts to collect ectoparasites other than ticks from the lairs of jackals were fruitless except that *Ctenocephalus canis* was captured on one occasion.

(2) INVESTIGATIONS ON THE TOXOPLASMA OF THE SPARROW.

Attempts to infect birds other than sparrows with *Toxoplasma* failed. Pigeons, fowls and crows proved refractory to this infection both when introduced by the mouth and by inoculation. This parasite appears to be specific for the sparrow. Examination of the blood showed the presence of the parasite in nine out of fifty-nine sparrows examined but evidence of infection was obtained in thirty-seven out of these fifty-nine birds at post mortem. Parasites appeared to be at highest concentration in the spleen. Segmenting forms resembling those described in *Plasmodium gallinaceum* were observed in the brain capillaries in a few cases but this requires further investigation. In the peripheral blood the parasites appear in the leucocytes. Material prepared for histological examination has not yet been studied in detail.

(3) INVESTIGATIONS ON THE HAEMOGREGARINE OF *Calotes. sp.*

Examination of mites (*Pterygosoma sp.*) parasitic on the lizard showed evidence of developmental cycle of haemogregarines. In fresh preparations cysts were seen in the body cavity which when burst (spontaneously or by pressure) were found to contain sporozoite-like forms which were actively motile. In serial sections of these mites, cysts and certain other bodies were observed, sometimes in great numbers. In some cases the appearances suggested the occurrence of an earlier developmental (precystic) stage but further observations are required on this point. The parasites in the peripheral blood were sheathed but in smears made

at post mortem from the internal organs it was observed that the parsites were detached from their sheaths which appeared as hyaline bodies after Romanowsky staining. The explanation of this observation requires elucidation.

(4) Investigations on the haemogregarine of the Gecko *Hemidactylus gleadovii*

Of sixty-nine lizards examined seven were found to be infected with a haemogregarine. Experiments to determine the transmitting agent gave negative results. Detailed investigation of prostigmatic mites (*Geckobia sp.*) did not show any evidence of the cycle of development of the parasite. Trials were also made with *Phlebotomus babu* but only a few could be induced to feed and these proved negative on dissection three days after feeding. Twelve specimens of *Culex fatigans* were also fed on infected lizards but all proved negative when dissected at intervals of from three to five days after feeding.

(5) Infectivity of *Leishmania donovani.*

Only twelve cases of kala-azar were available for study in Madras. The nasal secretions of these cases were examined for parasites and *L. donovani* was observed in one of them. Instillation of a preparation from the nasal secretion of this case was made into the nasal cavity of a Syrian hamster and this animal is now under observation.

(6) *Trypanosoma lewisi* in albino rats.

With a view to conducting experiments to determine the effects of well-balanced and deficient diets on the resistance of animals to protozoal infections, attempts were made to establish strains of *Trypanosoma lewisi* in albino rats. The results so far obtained are not in accord with those usually reported in the literature and the explanation of these findings is at present under investigation.

(7) Technique.

In the course of the work of the enquiry some interesting observations were made on the preparation of solutions for staining by the iron-haematoxylin method. It is stated in all standard reference works that it is necessary to prepare a fresh solution of iron alum from violet crystals. It was found, however, that equally good results could be obtained from old solutions or from those made from dirty yellow crystals, provided the haematoxylin used was of good quality. It is possible to obtain a clear solution of these crystals in half per cent. sulphuric acid and such solutions remain clear for prolonged periods and are as good for critical staining as fresh solutions prepared from violet crystals. The importance of these observations in protozoological work generally is obvious.

(8) Miscellaneous.

(i) Specimens of the tick *Haemaphysalis bispinosa* were bred and supplied alive to other workers in India and abroad. One consignment was despatched to Professor Brumpt in Paris where the ticks arrived in good condition and are now established.

(ii) Collections of various ticks and sandflies were made in the vicinity of Madras and subsequently identified.

(iii) Capt. Anderson, R.A.M.C., who is engaged in sandfly fever work under the Army Department received instruction on the methods of collecting, mounting and identifying sandflies and other routine technique.

(iv) Cultures of various blood flagellates were maintained.

47. Typhus enquiry by the Director, Pasteur Institute of India, Kasauli.

Three strains of typhus, two of the XK and one of the X19 serological types have been maintained during the year. They are now in their 65th and 166th generations, respectively. No changes in their characters have been noted. Using the methods practised originally a fresh strain of typhus XK was isolated from a patient this year and is now in its 8th generation.

The possible role of the bed-bug *Cimex lectularius* was studied in connexion with the transmission of typhus X19 (murine). Laboratory bred bed-bugs were fed on infected guinea-pigs and after a sufficient interval, emulsified and inoculated into clean guinea-pigs. A slight loss in weight and a rise of temperature to 103.4° F. was noticed in one animal out of five but no strain of typhus was recovered by inoculation of the spleen emulsion of this animal into others.

Typhus XK— reservoir of infection.

(*i*) *Hares.* This investigation was continued and a further series of 9 wild hares, *Lepus ruficaudatus*, shot in the endemic area around Sabathu were examined. No strain of typhus was obtained by the inoculation of the emulsion of their brains. It was also noted that no larval mites of the family trombiculidae were found on any one of the hares examined.

(*ii*) *Birds.* It was found that quails could be artificially infected with typhus XK, but as these are migratory birds it is unlikely that they have any connection with typhus in this area. Efforts to obtain young pheasants and jungle fowls which are present in this area throughout the year were not successful.

(*iii*) *Rats.* Fourteen wild rats and 3 shrews selected because they harboured large numbers of larval trombiculae were also tested for the presence of a typhus XK infection. Clean guinea-pigs were inoculated with spleen and brain emulsions. There was a reaction in three animals. In one of these the pathological features of ascites and marked enlargement of the spleen were present. Rickettsiae were also found in scrapings of the tunica vaginalis of this guinea-pig, denoting a typical infection with typhus XK. A rabbit inoculated with this strain gave a positive Weil-Felix agglutination with OXK to a titre of 1/1,000. The strain was, however, lost about the fourth generation as there was a complicating infection with *Spirillum minus*. It is possible, therefore, that here as in Malaya rats may serve as reservoir of the virus.

The ectoparasites from these rats including trombiculid mites, fleas and ticks were emulsified separately and inoculated into other guinea-pigs. In no instance was an infection obtained.

Transmission experiments of typhus XK with larval trombiculidae.

It may here be mentioned that repeated attempts to obtain larval trombiculae in sufficient numbers from the soil, vegetation and bird nests

by lavage or Tragardh's methods and also by the use of trap animals did not yield satisfactory results; the most fruitful source being the original one, viz., to obtain the larvae from the ears of wild rats.

Larval mites collected in this way were tested by the following methods:—

(i) Attempts to induce larvae to re-attach themselves on the ears of infected or clean white rats were not successful. It was found after many experiments that less than 1% of the larval trombiculae attached themselves for a second feed. This method for obtaining evidence of infection of trombiculae was therefore dropped.

(ii) The inoculation of large numbers of larval mites collected from rats into rabbits, guinea-pigs and monkeys did not produce any apparent signs of disease and the Weil-Felix reaction was also uniformly negative.

(iii) Rats found naturally infested with very young mites were inoculated with the laboratory strains of typhus XK and kept in captivity for as long a period as possible. The larvae were then collected, emulsified and inoculated into test animals. A rise in temperature up to 103.6° F. was noted in some guinea-pigs but other signs of typhus were lacking. The difficulty of keeping wild rats alive for a sufficient length of time in captivity appears to be one factor probably responsible for the negative results obtained. It has been ascertained that larval mites remain attached from 20 days to a month or more depending on the temperature before they are completely engorged.

(iv) Four monkeys were inoculated with emulsions of 26, 106, 198 and 96 larvae respectively. They showed no signs of illness and no significant Weil-Felix reaction was obtained.

Another factor which might explain the negative results obtained in the above experiments is the use of mixed batches of larval trombiculae. Out of the four species of *Trombicula* commonly found infesting wild rats only *Trombicula deliensis* is suspected to produce pathological results and these due probably to the abnormal monsoon conditions this year were not as abundant as in previous years.

It was reported last year that neither the tropical rat-mite *Liponyssus bacoti*, the tick *Hyalomma aegyptium*, nor the flea *Xenopsylla cheopis* can transmit typhus XK under experimental conditions. In view of these findings and the fact that *T. deliensis*, which is already suspected as the vector in Malaya, is found to attack man in the Simla Hills, it was decided that this potential vector be thoroughly studied. Breeding methods therefore were devised to obtain larvae in sufficient numbers for experimental purposes.

After several preliminary trials it was found that the best method to breed these mites consisted in keeping fully engorged larvae in small glass jars containing moist sterilized soil. The nymphal stages which were obtained in about two weeks at 24-25° C. were then transferred to large glass cages in which a variety of jungle plants were grown, thus imitating natural conditions. The soil in these cages was also kept sufficiently moist. The nymphs as far as could be ascertained do not feed on vegetable juices like those of an allied species *Trombicula akamushi* as observed by Japanese workers, but live on decaying animal and vegetable matter. In about ten weeks' time at 24-25° C. the nymphs metamorphose into adults. The first lot of laboratory bred larvae was observed three weeks after the

adults were first noticed in the breeding cage. The rate of oviposition appears to be very slow and it seems that the eggs are laid singly at short intervals. Only 10 larvae were obtained from two adults of *T. deliensis* confined in a separate jar during October and November.

Transmission experiments with laboratory bred larvae have therefore been undertaken. A wild brown rat and a white rat infected with one of the laboratory strains of typhus XK were kept in the breeding cage. After about a week 21 larvae were found attached on the ears of the brown rat only, of which 3 larvae appeared to be those of *T. deliensis* and the others *T. indica* (muris). After 22 days these larvae were removed from the ears of this rat, emulsified and inoculated subcutaneously into a rabbit. This animal reacted with loss in weight but the Weil-Felix reaction showed a slight rise of agglutinins for OX19. The results obtained so far have been encouraging and it is hoped to continue this work along these lines.

It has been emphasized by other workers on this problem that the breeding of these trombiculidae through their life-cycle in quantities sufficient for the carrying out of the transmission experiments, presents many difficulties, and much preliminary work will be necessary before such experiments will be possible. Having successfully worked out the life history of *T. deliensis* for the first time in India it is hoped to be able to conduct extensive experiments with the laboratory strains of typhus XK and determine the role of this mite in the transmission of typhus.

48. Filterable viruses enquiry under the Director, King Institute, Guindy.

1. Dengue fever virus.

The dengue fever virus has already been cultivated in this laboratory, on the chorioallantoic membrane. A small outbreak early in the year in St. Thomas Mount provided an opportunity for the further study of the virus. The sera obtained from typical cases during convalescence were studied in the usual manner. The virus was found to persist in blood for 40 days during convalescence. The presence of neutralising antibodies was also demonstrated by the usual technique. It was also found that the dengue fever convalescent serum did not neutralise the sandfly fever virus.

2. Researches on vaccinia virus.

Recent work on vaccinia virus particularly by Craigie & Wishart has revealed the fact that vaccinia virus material consists of two specific components viz., the elementary bodies and the specific soluble substance. These two components are readily separated from each other by ultracentrifugation and subsequent filtration through Seitz discs or appropriate collodion membranes. It has also been found that the specific soluble substance is a product of the elementary bodies.

Both the elementary bodies and the specific soluble substance have further been proved to consist each of a heat-stable and heat-labile constituent which produce specific anti-sera.

The heat labile constituent in both is inactivated by exposure to 56° C for one hour or to 1% formaline, while the heat-stable constituent withstands boiling and is unaffected by 1% formaline.

To start with; a strain of variola virus which after initial passage in monkeys had been stabilized by 42 pessages in calves was chosen for comparison antigenically and immunologically with vaccinia virus.

Calves were used both for the production of antisera as well as for virus materials. Calf scrapings taken on the 5th day are shaken well with citrate-disodium phosphate buffer solution. This material is then centrifuged at 3,000 R.P.M. for 5 minutes. The supernatant is preserved and the sediment is again mixed with more buffer solution and again centrifuged. The supernatant is mixed with the previous lot and centrifuged at 3,000 R.P.M. for 5 minutes. The final supernatant is centrifuged in an angle centrifuge at 3,500 R.P.M. for one hour. The supernatant is set aside and the sediment which consists mostly of elementary bodies is washed 3 times in the angle centrifuge with the buffer solution. Finally the sediment is suspended in a desired amount of buffer solution and centrifuged in the horizontal centrifuge at 3,000 R.P.M. for one hour. This sediments all the finer particles and bacteria which escaped the previous centrifugation in the same centrifuge. The supernatant is a pure suspension of elementary bodies and is treated with ether and then stored in cold. The first supernatant from the angle centrifuge when filtered through the Seitz disc gives the specific soluble substance. This for purposes of purification is first dialysed in cellophane bags and then all the protein material is precipitated at the iso-electric point of the material which is first determined. The pH of maximun precipitation of the antigen is determined by adding 0.2 c.c. each of Sorensen's HCL-citrate buffer, of pHs varying from 4 to 5, to 2 c.c. of the dialysed material and noting the test tube in which there is maximun precipitation. The pH of the buffer solution added to this test tube represents the pH of the solution to be added to the dialysate. This is then centrifuged and the deposit is recovered back into the solution by suspending it in Sorensen's citrate-NaOH buffer pH of 6.65. This is also treated with ether and then stored in cold.

Cross agglutination, cross precipitation and cross complement fixation tests reveal no differences between the passaged variola strain and vaccinia virus. Protection tests also confirm the above finding. Further tests with special regard to the heat-labile and heat-stable fractions are in progress.

A study of the antigens of vaccinia virus itself reveals the fact that infectivity and complement fixing property are intimately bound up with the 'L' factor.

49. Epidemic dropsy enquiry (laboratory section) under Major C. L. Pasricha, I.M.S. at the School of Tropical Medicine, Calcutta.

The laboratory studies carried out this year have given no evidence so far to associate any virus or bacteria in the causation of the disease. The association of mustard oil with epidemic dropsy as shown by Dr. R. B. Lal was studied. In the work there was close collaboration with Col. R. N. Chopra and the enquiry was strengthened by the association of Dr. R. K. Goyal of the endowment fund appointed especially to study this aspect of the problem and valuable help was given by Dr. A. K. Sen of the Bengal Public Health Laboratory in the testing of implicated samples. It was found that argemone oil (oil from the seeds of *Argemone mexicana*) was present in mustard oils associated with outbreaks of epidemic dropsy. Pure mustard oil to which oil expressed from *Argemona mexicana* seeds was added produced oedema, cardiac involvement in human volunteers to whom this oil was given. Another series of volunteers, who received mustard oil which had been used by individuals subsequently developing

clinical epidemic dropsy, gave similar results. This latter oil gave marked reaction for argemone oil. A summary of the result is given in tables A. and B. The amount of argemone oil present in implicated mustard oils was estimated (calorimetrically) to be about 5 to 10 per cent. The test for this oil is a simple one and consists in the addition of nitric acid to mustard oil in a test tube. The tube is shaken and a positive reaction is shown by the development of a brown to an orange-red colour in the nitric acid.

Two groups of persons took food cooked in oil incriminated in outbreaks of the disease and pure mustard oil to which oil expressed from *Argemone mexicana* seeds had been added. The results are summarised in tables A. and B.

In one subject in each group there was transitory diarrhoea. In the group in which oil containing argemone oil was used for cooking, granular or hyaline casts were found in three out of the five persons but without any demonstrable albumen. In this connection it may be noted that granular casts were found in 7 out of 16 epidemic dropsy patients examined.

Two individuals one in each group complained of dimness of vision but when examined showed no evidence of glaucoma.

Two of the four individuals who ate food cooked in incriminated oil developed oedema of the legs. In one the use of incriminated oil was stopped owing to intercurrent fever. In the fourth subject although the incriminated oil was used for 21 days there were no ill effects. This individual was the only non-Bengalee of the series and ate a limited amount of rice, eating instead bread (as 'chapaties').

The five individuals whose food was cooked in mustard oil containing known quantities of argemone oil developed well marked oedema. Two of them (A 3 and A 4) in addition had flush and cardiac symptoms.

Food cooked in oil containing 5 per cent. argemone oil was apparently relished but the food cooked in 10 per cent. argemone oil soon led to loss of appetite.

It appears that the principle or principles present in argemone oil have a cumulative effect and provided sufficient quantity of the oil is consumed, symptoms appear after an interval even though the consumption of argemone oil or incriminated oil is stopped. This fact must be borne in mind in any investigation of mustard oils in outbreaks of the disease. The oil used at the time of the outbreak can be pure and innocuous but it is the oil used some days before the outbreak which must be examined. Positive reactions for argemone oil were obtained in the majority of the samples of oils collected from houses where there had been cases of epidemic dropsy. Four of the 28 samples collected in the open market gave positive reaction.

A portion of the skin removed from one of the individuals on food cooked in oil containing argemone oil and who developed flush and oedema showed marked dilatation of the capillaries with round cell infiltration and the presence of fibroblasts. The changes in the skin were similar to those observed in the skins removed from five epidemic dropsy patients. Similar capillary dilatation was noted in the tissues and organs (skin, liver, kidneys, spleen, lungs and heart) of cats which had been given either heated or unheated argemone oil. In this connection it must be noted that similar capillary dilatation was found in the skin and organs of a young pig

injected with massive doses of a culture of a gram positive aerobic-spore-forming bacillus isolated from dust. This opens up a vast subject for investigation and diminishes the importance that is attached to the generalised capillary dilatation that occurs in epidemic dropsy. Animal feeding experiments in the laboratory are in progress.

Two alkaloids, berberine and protopine have been isolated from *Argemone mexicana* plant. It is known that berberine even in large doses is not toxic to man. The action of protopine has not been fully studied. Whether this alkaloid or any other substance is responsible for the toxic effects remains to be determined. The outstanding facts are :—

(1) Argemone oil when present in oil used for cooking gives rise to certain symptoms. The most marked effect is oedema of the legs and in some the development of a flush and cardiac involvement. Similar symptoms are produced by the use of mustard oil which is implicated in outbreaks of epidemic dropsy.

(2) Whether the condition produced is identical or not to the naturally occurring epidemic dropsy cannot be definitely said at present but there is no doubt that the use of oil containing argemone oil is harmful to man. There appears to be little doubt that consumption of adulterated oil produces symptoms which cannot be distinguished from those occurring in this disease.

(3) From the evidence available it appears that the adulteration of mustard oil with argemone oil is not intentional on the part of those who grow the mustard plant or of those who express or sell the oil. The plant *Argemone mexicana* appears to be widespread and present in many mustard plant fields and its seeds are harvested along with mustard seeds.

During the course of virus work in connection with epidemic dropsy it was found that material obtained by sternal puncture produced lesions on the chorio-allantoic membrane of chick embryo. These lesions were passaged eight times. Similar lesions were produced by material collected from non-epidemic dropsy individuals.

The fluid removed by Col. Kirwan from the anterior chamber of the eye of epidemic dropsy patients suffering from glaucoma is being studied for the presence of a virus.

The sedimentation rate of red blood corpuscles, the haemoglobin and cell volume were estimated in 58 cases of epidemic dropsy and 24 healthy individuals. The results are :—

		With marked oedema.	With slight oedema.	With no oedema.
Epidemic dropsy patients ..	Males	107	59	38
	Females	83	64	
Individuals who took food cooked in oil containing argemone oil.	Males	106	54	39

The average normal sedimentation rate (1 hour) was found to be 12 m.m. There is an increase in the sedimentation rate when oedema is present. As the oedema disappears the sedimentation rate becomes less.

50. Epidemic dropsy enquiry (field section) under Dr. R. B. Lal at the All-India Institute of Hygiene and Public Health, Calcutta.

These were carried out in seven different centres in Bengal. The primary object of these investigations was to collect samples of mustard oil, incriminated on epidemiological grounds, for detailed chemical and other investigations. Such oil was recovered in sufficient quantities from two places, viz., Rangpur and Alamdanga. These two supplies were tested for potency by human feeding experiments. With the Rangpur oil, two out of three volunteers showed definite symptoms of the disease. With the Alamdanga oil, only one suspicious case developed in a group of 3 volunteers. All the six volunteers on the test oils developed some premonitory symptoms, viz., diarrhoea, fever, etc. Definite symptoms of the disease developed in all the three persons taking Rangpur oil and in one out of the three who had been put on Alamdanga oil. The three controls taking the jail-produced oil remained healthy throughout. The administration of the oils was stopped as soon as the object of the experiment was served. A section of skin from the leg of one of the experimental cases was examined histologically. The pathological changes observed were similar in nature, though not advanced to anything like the same degree as seen in skin sections obtained from post-mortems of cases of epidemic dropsy which were used for comparison.

About 300 samples of mustard oil were obtained from different places where outbreaks of epidemic dropsy had been reported. The epidemiological histories of the samples were recorded in a standard form. On the basis of the information thus supplied, each sample was classified according to its degree of association with the disease. With the help of this material endeavours were made (*i*) to develop differential tests for potent mustard oils, and (*ii*) to determine the nature and origin of the deleterious substance in the poisonous oil.

Differential tests.

These may be classified under two heads :—

(*a* Biological and
(*b*) Chemical and physical.

Biological tests.—These tests consisted of :—

(1) feeding experiments on rats, cats, guinea-pigs, swine, and monkeys.

(2) intracutaneous inoculation in human subjects. The experiments on animals are still in progress and will be reported later on. The skin tests which were pursued for a month failed to give positive results.

Chemical and physical tests.—(1) The usual physical and chemical constants required under the Bengal Food Adulteration Act of 1919 were determined. The proved potent oils showed no evidence of adulteration.

(2) A large number of reagents were employed to test for other possible adulterants of mustard oil, and for any other reactions which might prove differential. It was found that the potent oils possessed a greater reducing property than the non-potent oils. With regard to other tests, the results were negative except with Crace Calvert's and Cavilli's reactions for non-drying oils. On shaking samples of potent oil with these reagents and allowing the mixture to separate, an orange-red colour was observed in the bottom layer. No such colour developed with the

nonpotent oil. It was noted that nitric acid was a common constituent of these two reagents.

(3) Hauchecorn's test which consists of vigorous shaking of a little quantity of oil with an equal volume of concentrated nitric acid was next performed. A brownish red colour developed in the bottom acid layer with the proved potent oil, whereas this acid layer remained quite clear with a known non-potent oil, thus suggesting a differential test between a potent and a non-potent oil.

(4) Another test, which was tried at the suggestion of Dr. S. S. Bhatnagar and found differential was the addition of a small quantity of a 3 per cent. solution of cupric acetate to an equal quantity of test oil acidified strongly with glacial acetic acid. On heating, a precipitate formed and the colour of the watery layer changed from blue to green in case of the potent oil, but not in that of a non-potent oil. Since cupric acetate test was believed to be a specific reaction for mercaptan and other organic sulphides, small amounts of ethyl mercaptan and allylisothiocyanate were added to jail-produced oil for purposes of comparison. The mixture however failed to give the typical positive reaction with nitric acid.

(5) The two chemical tests mentioned above were applied to the 300 odd samples of mustard oil previously referred to. The results of the tests closely followed the epidemiological histories of the different samples. These simple reactions could, therefore, serve as differential tests.

(6) Side by side with the chemical tests, spectro-photo-metric studies by the absorption method were made on samples of oil in alcoholic solution. The general nature of the absorption curve was similar in all cases, but the sample of proved potent oil showed a broad absorption band between 2900 A. U. and 2600 A. U. with a maximum near about 2750 A. U. Further work suggested that the specific absorption could also be used as a differential test.

(7) A violet fluorescence is seen when the potent oil is exposed to light. This is not observed in the case of non-potent oils. These differential tests constitute important development in the history of investigations on epidemic dropsy, because it is now possible to apply simple tests on samples of oil in the field. The consumption of the supplies giving positive reactions could be stopped, and further development of cases prevented. Thus these differentiating tests place in the hands of the public health authorities an important weapon for fighting the disease.

Source of the poisonous substance in the oil.

For an effective control of the disease it is desirable to find out the source from which the poisonous substance in the supplies of mustard oil is derived. There are two possibilities, *viz.*, (i) deterioration of mustard seeds on account of faulty storage and infection with some fungoid or other forms of organisms with consequent elaboration of toxic factors. (ii) (*a*) adulteration of the oil with some cheap poisonous oil, (*b*) admixture of mustard seeds with some poisonous seeds either deliberately or inadvertently.

These possibilities have been investigated with the following results :-

i. *Deterioration of mustard seeds.*

A quantity of mustard seeds which had been apparently damaged by fungoid growths was obtained. Oil was expressed from these seeds and was tested by means of feeding experiments on human volunteers. The

general outline of the experiment was the same as reported previously. No ill effects were, however, observed in any of the experimental subjects.

ii. *Use of adulterants* :—

(*a*) adulteration with some oil :—This has been discussed above.

(*b*) adulteration of mustard seeds with other seeds.

The observation that millowners themselves very often suffered from the disease suggested that the source from which the oil derived its poisonous substance was unknown to them. The possibility, therefore, of the seeds themselves being mixed either deliberately or unknowingly with some poisonous seeds resembling mustard seeds was investigated.

Taking the lead from two published reports, the seeds of *Argemone mexicana* were suspected. The oil expressed from these seeds was examined and it was found that a mixture of 5 to 10 per cent. of this oil with jail-produced mustard oil satisfied the same physical and chemical tests as the known potent oil.

Samples of mustard seeds were obtained from the stocks of 40 oil mills in and around Calcutta. Seventeen samples contained *Argemone mexicana* seeds. A further experiment was performed which showed that the source of the substance giving the differential tests could be traced to the seeds of *Argemone mexicana* and not to seeds of other plants found in the mustard seeds. Thus while the oil pressed in the laboratory from a supply of seeds containing 10 per cent. *Argemone mexicana* seeds gave strongly positive differential tests, the oil pressed from the same supply after the *Argemone mexicana* seeds had been removed failed to give these reactions.

These observations suggest that the poisonous substance in the oil causing the symptoms of epidemic dropsy is derived from the seeds of *Argemone mexicana* which grows wildly and plentifully in various parts of India, and gets mixed with the mustard seeds in varying proportions at the time of harvesting. The other possibility is that these seeds are deliberately used as adulterants by the dealers.

NATURE OF THE TOXIC SUBSTANCE.

A white crystalline substance has been isolated in pure form from the oil of *Argemone mexicana* which gives all the differential physical and chemical tests in high dilutions. A similar substance has been recovered from Rangpur oil. It could not be recovered from oil pressed from mustard seeds freed of the *Argemone mexicana* seeds. While the crystalline substance is not soluble in water, it is soluble in organic solvent like ether, alcohol, chloroform etc., and in acidulated water. It is an organic compound. Work on the micro-chemistry and chemical structure of this substance is in progress.

While it has been possible to track down the chemical substance which is derived from *Argemone mexicana* oil and which satisfies the differential tests it does not necessarily follow that this substance is responsible for the symptoms of the disease. Further work on this aspect of the problem is in progress.

51. Kala-azar continuity enquiry by Dr. L. E. Napier at the School of Tropical Medicine, Calcutta.

This small enquiry with personnel consisting of a research worker of the sub-assistant surgeon class, an insect collector and a laboratory

attendant, is the residue of the large kala-azar commission and the kala-azar enquiry based at the School.

Two kala-azar centres are maintained, one at the School and the other at a village near Calcutta. The latter is in the area that has been kept under observation for a number of years; at the height of the kala-azar 'epidemic' there were 137 cases in one year from the survey area, last year there were none, and this year there are four. It seems possible that another wave of kala-azar is coming.

Studies in the bionomics of sandflies are being continued in this area. Figures for the incidence and survival rate of flies have been collected for another year. The seasonal curve seems to be the same as last year, so that it may probably be shown that the variation from month to month is a truly seasonal one and not due to chance.

Further, therapeutic trials have been carried out with solustibosan, a drug that was mentioned last year, and a new pentavalent compound from Messrs. Parke, Davis & Co., has now been received and is also being tested.

Sternum puncture has been found to be a very reliable method of diagnosis, only just short of spleen puncture in the matter of accuracy, and possibly safer.

A sub-assistant surgeon who had been posted to work in Sikkim came here for training. In connection with the outbreak of kala-azar in Sikkim Dr. Napier has kept in close touch with the Resident of Sikkim and with the chief medical officer. In connection with the kala-azar campaign in Bihar, the Inspector General of Civil Hospitals has arranged a conference with Col. Chopra and Dr. Napier.

These last facts are mentioned as they demonstrate the importance of maintaining an enquiry under the I.R.F.A. on this important subject.

52. Investigations of toxicological and medicolegal importance by Rai Bahadur Dr. K. N. Bagchi, Chemical Examiner to the Government of Bengal and Professor of Chemistry, Medical College, Calcutta.

Lead-content of normal human tissues.

During the period under review, an investigation into the lead-content of normal tissues was continued and about 150 specimens covering all sorts of tissues were analysed with a view to finding out a limit for lead in healthy Indians. The materials for examination were supplied by the Police Surgeon of Calcutta and the medical officers in charge of sub-divisions and districts of Bengal, Bihar, Orissa and Assam. Attempts were made to make the selection of materials as representative as possible. No hospital cases nor any case with a history of terminal illness in which partial starvation, disturbed metabolism and other factors interfere with the normal distribution of lead in the tissues, were included in this investigation. Only cases of rapidly fatal street accidents, shooting, stabbing, etc. were selected for collection of materials. As the viscera of about 900 individuals are received annually in this laboratory for medicolegal analysis, there was no dearth of suitable materials for this enquiry.

The result of this investigation shows that the lead-content of normal tissues of Indians is much less than that of the Europeans. The small amount of lead present in food and water and also the simpler conditions of life prevailing in this country in comparison with those in the highly advanced and industrialised cities of the West, offer less opportunities for lead absorption. The following figures (milligram per kilo) showing the maximum amount of lead in some of the healthy tissues are of interest—

Liver	0.82 mg.,	Kidney	0.71 mg.	Spleen	0.52 mg.
Stomach	0.6 ,,	Intestines	0.68 ,,	Lungs	0.60 ,,
Blood	0.45 ,,	Brain	0.10 ,,	Bones	39.3 ,,
Tooth	23.0 ,,	Hair	130.0 ,,	Uterus	0.47 ,,
Ovary	nil	Testicles	0.40 ,,	&c.	&c. &c.

The absence of lead in the ovaries of healthy women (six specimens examined) is significant. In one case (with history of exposure to lead)—the ovaries contained only 0.17 mg. of lead. It is known that women workers in type foundries and other industries connected with lead get frequent miscarriages. This fact along with the absence of lead in ovaries of women not exposed to lead and presence of lead in ovaries of those exposed to lead, lends support to the theory that lead causes damage to the fœtus and germ cells and thus produces sterility and miscarriages. It is not understood why testis behave differently.

Five fœtuses of different ages were examined. The result shows that they have no special affinity for lead.

Hair presents a most remarkable phenomenon. Although a dead tissue, it retains a large quantity of lead. The fact that hair is rich in lead has not been observed by any European or American worker. It appears from the result of analysis of 55 samples of hair collected from among Indians, Anglo-Indians and Europeans that lead plays some part in producing the pigments of hair which characterise different nationalities and races. The black hair of Indians, specially of Indian women, contains the maximum amount of lead (130 mgs.) while golden, brown and grey hair contains much less (32 mgs.). This portion of the investigation is in progress, and will be conducted on a more comprehensive scale. The result of analysis of other tissues is ready for the press and will shortly be sent to the Indian Journal of Medical Research for publication.

(2) Lead in common foodstuffs and water.

About 30 samples of food and water have been examined for their lead-content. Rice, potato, onion, radish, pumpkin and many food materials of vegetable origin contain no appreciable amount of lead while milk, fish, meat, and some fruits (orange, mango, guava, etc.) and tea contain lead varying from 0.1 to 0.6 mg. per kilo. The lead-content of tap water of Calcutta and also of well and tank waters of the suburbs does not exceed 0.002 mg. while the tap water of Glasgow, for example, contains as much as 0.03 mg. (15 times approximately) of lead per litre. These figures clearly indicate that the lead-intake of an Indian is likely to differ widely from that of a European and therefore the concentration of lead in the tissues will be correspondingly less. Kehoe and other American workers have shown that after a fairly definite concentration of lead in the tissues is reached, lead-intake and lead-elimination show a close agreement. The lead-elimination of Indians has already been shown to be at much

lower level (Bagchi & Ganguli, Ind. Jourl. Med. Res. July, 1937). Many more samples of foodstuffs will be examined for further information.

(3) Hydrocyanic acid in linseed flowers.

In connection with an outbreak of cattle poisoning which resulted in the death of a number of bullocks and cows, hydrocyanic acid was detected in the stomach contents and viscera of some of them. The source of this poison was traced to feeding on the flowering tops of the common linseed plant (*Linum usitatissimum*). The different parts of the plant were analysed and it was found that flowers with immature seeds contained the maximum amount (0.69%) of hydrocyanic acid. Mature seeds (when ready for harvesting) contained 0.08% and linseed cake contained 0.032% of the acid. The maximum yield of hydrocyanic acid is obtained from the flowers (containing the cyanogenetic glucoside—*linamarin*) if they are soaked in water for about 4 hours before distillation. Acids and alkalies retard hydrolysis of the glucoside and prevents the formation of hydrocyanic acid. A strong solution of an alkali has therefore been suggested as an antidote in linseed flower poisoning which is fairly common in Bengal, Bihar and other linseed growing provinces. The result of this investigation has been sent to the Indian Journal of Veterinary Science and Animal Husbandry, for publication.

(4) Copper-content of normal human tissues.

This investigation has also been continued this year. 23 samples of normal tissues have been analysed with the following result. The figures indicate an average in milligramme per kilo—liver—10.8 mg., kidney—10.5 mg., lungs—6.25 mg., spleen—5.75 mg., stomach—6.75 mg., heart—3.5 mg., etc. etc.

The other tissues will be analysed in due course. As fatal cases of copper sulphate poisoning are fairly common in Bengal, a correct idea as to the limit of copper in normal tissues is essential. Knowledge of the copper content of human tissues is also of biochemical importance.

Fœtal tissues show a special affinity for copper. The liver and kidney of a seven months' fœtus gave 37.0 and 12.5 mgs. of copper respectively and in a case of copper sulphate poisoning only 37.5 mgs. (per kilo) of copper were found in the liver. This appears to be anomalous and further investigation is proceeding. It is interesting to note that in cases of acute arsenic poisoning one finds as much as 500 mgs. or more of arsenic (as As_2O_3) per kilo of liver (Bagchi and Ganguli. Ind. Med. Gaz. 1937) but in acute copper or lead poisoning cases such large quantities of the poison are not found in any of the tissues. The quick and selective absorption of a large quantity of arsenic by the liver and other tissues and their capacity to retain and fix it up in the tissues are probably the factors which account for rapid death in arsenic poisoning. Lead and copper on the other hand are slowly absorbed and quickly eliminated and death usually takes place after a few days as the result of complications arising from damage caused to the organs.

This investigation will be continued further for determination of the copper content of all the tissues of the body and also of faeces and urine of normal persons.

53. Studies on bone-marrow, etc. by Dr. H. N. Chatterjee at the Carmichael Medical College, Calcutta.

Various post-mortem specimens have been secured from the Carmichael Medical College and the Medical College, Bengal.

The specimens of bone-marrow have been secured for histological examination from the following cases :—

Cholera, kala-azar, enteric fever, epidemic dropsy, Bengal splenomegaly, cancer & malignant disease, malaria, secondary anaemias, anaemia of pregnancy, myeloid leukemia, haemorrhageic deathesis, diptheria, pulmonary tuberculosis and other cases.

The number of specimens secured during the current year is 60 and that of the total number secured so far is 105.

The specimens are being studied.

Besides the above, intact specimens of femur have also been secured and are being preserved in the museum of Carmichael Medical College; this study of these macroscopic specimens will be a valuable adjunct to the histological study.

As bone-marrow is very sensitive to various types of infections and physiological conditions, the work which has already been done by the enquiry and that which is to follow is likely to enhance knowledge not only on that comparatively neglected and obscure structure but also on the underlying pathology of the different conditions.

As an example, the bone-marrow findings in cholera have revealed many facts which are likely to greatly modify our knowledge on the subject. These findings are being presented before the 26th session of the Indian Science Congress.

A similar study of the bone-marrow of kala-azar had previously revealed a great deal of information as to the underlying pathology of the blood changes of kala-azar in different stages of the disease.

54. Enquiry into secondary anaemias by Dr. H. N. Chatterjee at the Carmichael Medical College, Calcutta.

It has been possible to perform a complete haematological examination on cases of anaemias of various causes and types. In many cases the observations were often repeated. In all cases the items of examination included haemoglobin, total white and red blood cell counts, reticulocyte count, Schilling differential count, corpuscular volume (haematocrit) halometer index Price Jones Curves of red and white cells, mean corpuscular volume and haemoglobin, serum albumin and globulin, blood cholesterol, Van den Bergh and the different indices. The cases examined since the corresponding report of last year was sent are 67 in number making the total number of cases examined so far 142. These include the following cases :—

Chronic malaria, kala-azar, chronic septic infection, haemorrhage, helminthic, infections, chronic enteritis, chronic gastric troubles, malignant growth, pulmonary tuberculosis, black-water fever, pernicious anaemia, cirrhosis of the liver, epidemic dropsy, anaemia following drugs.

It would thus be seen that a good deal of material has accumulated and if followed up, this material is likely to furnish valuable information about the haematology of different types of secondary anaemias in this country.

Besides the above, in the last year's report, a programme was presented for finding out the euglobulin and pseudoglobulin factors as well as the ester and free cholesterol fraction in the blood of cases of idiopathic anaemia of pregnancy and normal pregnancies.

This investigation has been carried out and the findings have been presented in a paper to be read before the 26th session of the Indian Science Congress at Lahore.

Therapeutic investigations have also been carried out on laboratory animals (white rats) based on the biochemical findings mentioned above. The same investigations have been and are being followed up in cases of these type of anaemia. The results are encouraging.

Therapeutic investigations were also studied in the anaemia of pregnancy with the different types of treatment and the results have been published in the October, 1938 issue of the Journal of the Indian Medical Association.

55. Thrombo-angeitis obliterans enquiry by Dr. R. N. Cooper at the Seth G. S. Hospital, Parel, Bombay.

This disease has for a number of years passed unnoticed. The terminal manifestation of this disease is a variety of dry gangrene. Limbs were amputated and the diagnosis are put down as dry gangrene. The amputated limbs were thrown away. The present enquiry was instituted to study this disease in great detail.

The object of the whole enquiry is :—

(1) to study critically the clinical manifestations of the disease,

(2) to assess the value of various exciting factors such as climate, occupation, habits of life such as smoking etc.,

(3) to estimate the extent of disease of the blood vessels by

- (*a*) digital palpation of terminal arteries
- (*b*) postural colour changes
- (*c*) recording of surface temperature of extremities by a thermo-couple
- (*d*) recording the pulsation of blood vessels by an oscillometer
- (*e*) arteriography
- (*f*) opthalmoscopy

(4) to make a complete study from the point of view of pathology

(5) to assess the value of various therapeutic methods.

With these objects in view during the year 29 cases were investigated. Of these 25 were fresh cases and 4 were cases which were previously treated and returned for further treatment. Of the 25 fresh cases, two were cases of ainhum and one of femoral aneurysm with intermittent claudication.

(1) A CRITICAL CLINICAL STUDY.

It was realised that cases could not be studied unless a definite scheme for investigation was formulated. Accordingly a detailed history of the case is obtained according to a prepared plan which embraces the various aetiological factors concerned. Appendix (A) will show the type of questionnaire which was prepared and modified by subsequent experience.

This scheme focuses one's attention to the early symptoms. Thus every case of tired-feet simulating flat-feet deserves a careful investigation. Similarly many a pain labelled as sciatica has been found to be of vascular origin. One has noticed a case being subjected to non-operative nerve stretching for supposed sciatica with precipitation of gangrene. Gluteal pain after exertion requires careful investigation.

Many clinical types of this disease have been described in monographs on this subject. Routine examination has enabled one to record an unusual manifestation of an acute onset, with profound muscular atrophy and marked weakness in the extremity simulating palsy.

The investigations are yet incomplete. Further investigations are necessary to collect sufficient data under various aetiological, diagnostic and therapeutic heads.

(2) ASSESSING THE VALUE OF VARIOUS EXCITING FACTORS.

Further investigations are necessary. It is proposed to send out the questionnaire to various leading hospitals of India. Co-operation from these hospitals will enable one to form some estimate of the influence of climate, seasons etc. Personal records show that the number of cases fell off considerably between the months of February and July. It is curious to observe that not one of the investigated patients belonged to the Jewish race.

(3) ESTIMATION OF THE EXTENT OF ARTERIAL DAMAGE.

The extent of arterial disease can be estimated in various ways as mentioned above.

(*a*) *Digital palpation.*—By digital palpation one detects the presence or absence of pulsation in various terminal arteries. This clinical observation is easy and accurate. But the absence of pulsation may still be compatible with an efficient co-lateral circulation. And prognosis of the case depends on the efficiency of the co-lateral circulation. This can be estimated by other means.

(*b*) *Postural colour changes.*—In this disease colour changes such as cyanosis and pallor are influenced by posture. A limb hanging down is cyanotic. If however the ankles are moved up and down, the colour begins to fade in the case of the limb which is affected with arterial disease. The colour changes are best noticed when the lower extremities are held up vertically. This is a fairly delicate test in white skins. In our country this test has its limitations.

(*c*) *Surface temperature records.* — These are obtained by a thermocouple. Interdigital temperature is recorded in both the upper and lower extremities in each case. Room temperature is also recorded at the same time. Improvement obtained by any therapeutic measure is thus observed objectively.

(*d*) *Oscillometry.*—This is done by a Pachon type of oscillometer. This is a very delicate test. The amplitude of the oscillations is graphically recorded at the level of the wrist, the arm, the ankle and the thigh. These records are particularly valuable in assessing the success of any therapeutic measure.

(*e*) *Arteriography.*—This is obtained by the injection of Uro-selection B in a restricted field of circulation. In the beginning it promised to be of great diagnostic and prognostic help. It delineates with great precision the exact site of the disease, its extent, and the extent of new co-lateral circulation opened up. It enables one to judge accurately whether an amputation would be necessary. However one soon learnt the limitations of this method of study. In every case there was severe pain complained of in the limb. In one case while investigating the lower extremity, one noticed an oedema of both the lower limbs as high as the knees soon after the injection. The patient did not have co-existing cardiac or renal disease. The oedema passed off. In another case, gangrene was precipitated by the injection and amputation had to be resorted to.

(*f*) *Opthalmoscopy.*—As the vessels of the eye are so readily observable in life a thorough study has been made of these cases from that point of view. The results so far do not show any consistent variations from the normal.

(4) Pathological study.

Pathological investigations were undertaken along various lines.

Pathological studies make one suspect that the disease is essentially infective in origin. The infection may be an organism or a virus. Some workers in America have isolated an organism. This has not been confirmed. Our attempts to recover an organism from blood, or from freshly excised thrombosed superficial veins have been unsuccessful. Attempts have been made to obtain a filtrate both from a vein and an artery. These have also failed.

A detailed study of the various arteries of the body was made in one case which died. The disease was not limited to the arteries of the extremities. Various visceral vessels were involved. These changes in the vessels may explain some of the unusual symptoms—abdominal, cardiac, cerebral, or genital complained of by the patient s. Further observations of this nature will be very helpful.

A routine investigation of arteries, veins, lymphatics and nerves of amputated limbs is made. During an operation of lumbar sympathectomy one often notices enlarged lumbar glands. These glands also have been purposely excised, and investigated from bacteriological and histological standpoints.

These routine examinations have brought out some interesting points. In one case, the nerve showed the existence of a nodule of leprosy. This patient did not show clinically any sign of Hansen's disease. It is proposed to investigate histologically the arterial condition of an amputated limb of a leper.

The lymphatic glands are enlarged and show an increase in the amount of reticular tissue both in the cortex and the medulla. Attempts to obtain any organism from these glands have failed so far.

From the beneficial therapeutic effects of hypertonic saline it was decided to investigate the blood-chlorides and the excretion of chlorides in the urine. No investigations of this nature have been recorded so far in published monographs. The results obtained are interesting. An appreciable number amongst the investigated cases show a diminution of blood-chlorides. The normal figure for blood-chlorides is 450—500 mgm. The lowest reading has been 363 mgm. Nearly fifty per cent. of the cases show a figure between 390 and 430 mgm. Hypertonic saline treatment was particularly beneficial in cases with a low blood-chloride.

The results of urine-chloride excretion are not yet scrutinised sufficiently to give any information of value.

Believing that biochemical investigations were useful, it was decided to estimate the blood cholesterol.

Blood sedimentation rate is also studied. These investigations have been just commenced. Only three cases have been examined so far.

Vitamin C plays an important part in infections. It was decided to try the vitamin C excretion test in the urine. Five cases were investigated. These results will be communicated later.

(5) ASSESSING THE VALUE OF VARIOUS THERAPEUTIC MEASURES.

Oscillometric records are very interesting. Buerger has advocated postural exercises. Oscillometric records taken in different positions have enabled one to understand the rationale of the treatment.

The success of any line of treatment is to be measured by (*a*) the relief of symptoms (*b*) the healing of ulcers and gangrenous areas (*c*) the amplitude of the pulse and (*d*) the rise in the surface temperature of the extremities. Judged from these points of view all therapeutic measures are disappointing.

At one time arterial symphatectomy and lumbar ganglionectomy were expected to be a big advance in therapy. The study of the 'relapsed cases' shakes our faith in the permanency of results obtained by surgical measures. The disease may end spontaneously. However in a majority of cases the disease is progressive and multiple amputations are not uncommon.

The value of hypertonic saline is definitely proved by the relief that is obtained from pain even after a single injection. In most of the cases the injection is to be repeated. 300 c.c. of 4% solution repeated every 4th day seems to be adequate for most of the cases. The value of saline treatment is again seen in the arrest of gangrene and the early establishment of the line of demarcation.

The graphical records by the oscillograph before and after injection of hypertonic saline are interesting and enable one to assess the probable value of this treatment.

The whole problem is like a jig-saw puzzle. It will take more prolonged investigation to see how the different pieces fit into the final picture.

Appendix 'A'

Scheme of line of investigation in a case of Thrombo-angeitis obliterans.

Age.....Occupation.....

Nationality.....Religion.....Birth Place.....
(Climatic conditions thereof)

.....Time of year when patient first reports for treatment.....

Family History (any vascular disease in the family also T. B. and Leprosy).

Tracing a family tree of vascular disease, if possible.

Personal History—smoking.

Symptoms :—

First symptoms to appear and precipitating factor *e.g.*, cold, injury, undue exertion and sequence of symptoms :—

(1) Burning sensation in sole of foot after exertion.
(2) Numbness in various parts of extremities.
(3) Subjective coldness.
(4) Abnormal fatigue.
(5) Claudication, situation, severity and progress.
(6) Pain in buttock after exertion which radiates into the thigh like sciatic pain.
(7) Rest pain, if any, especially in upper extremity.
(8) Colour changes dependent on posture.
(9) Vaso-motor disturbances *e.g.*, sweating or its absence.
(10) Superficial phlebitis, situation, recurrence.
(11) Cutaneous nodosities or unusual dryness.
(12) Malformations of nails.
(13) Atrophy of muscles.
(14) Ulcers and gangrene.

Disability and economic loss.

Previous diagnosis.

Previous medical treatment.

Any surgical procedures previously adopted (giving main details of the procedure adopted and results).

Clinical investigation :-

Complete vascular and neurological picture *e.g.*,

1. Clinical estimate of extent of potency of big peripheral vessels *e.g.* femoral popliteal anterior and posterior libial auxillary, brachial and radial as revealed by
   (*a*) digital palpation
   (*b*) study of surface temperature by a thermo-couple
   (*c*) colour changes as induced by posture
   (*d*) oscillometry by Pachou oscillometer of specified arteries.

2. Presence of sensory and motor changes in extremities.

Routine search for infective focus.

Teeth
Tonsils
Antra
Urine
Faeces.

The last two being laboratory examinations.

3. When claudication appears on muscular exertion (in minutes) *e.g.* movement at ankle or walking (before and after treatment).

Routine exclusion of :—

1. Glycosuria, if even a trace, blood sugar curve.
2. Wasserman and Kahn. Reaction.
3. Leprosy, clinical examination and pathological.
   (*a*) post-nasal swab.
   (*b*) skin section.

Special investigation of vaso-spasm :—

1. Vaso-motor index with cu-constantan couple by
   (*a*) general pyrexia (T. A. B. vaccine).
   (*b*) nerve trunk injection by a local anaesthetic
   (*c*) spinal anaesthesia.

Bio-chemical investigations :—

(*a*) blood-chlorides
(*b*) blood cholestrol
(*c*) vitamin C excretion test
(*d*) blood sedimentation rate.

Biopsy of veins from suspected cases :—

(*a*) section
(*b*) culture
(*c*) innoculation

Post-mortem investigation of all blood vessels of patients from 30 to 45 years.

Diagnostic and therapeutic :—

3% to 5% saline — 100 c.c. weekly.

Ophthalmic report of every examined case to be appended especially with regard to the condition of the retinal vessels, existence of retinitis pigmentosa etc.

Treatment adopted and results evaluated *e.g.* measure of relief obtained.

(*a*) subjective improvement.
(*b*) objective improvement.

Treatment adopted :—

(*a*) purely medical
(*b*) postural
(*c*) physiotherapeutic *e.g.* contrast baths, diathermy
(*d*) surgical.
   1. Periarterial sympathectomy.
   2. Ganglionectomy.

An attempt at formulating a prognosis.

56. Oroxylin enquiry by Prof. A. S. Paranjpe at the Seth G. S. Medical College, Bombay.

Oroxylin was extracted from the root bark of Oroxylum Indicum Vent., which is one of the roots in the "Ten roots" (Dashamoola) preparations in Ayurveda. The root bark is described to have the following actions and uses in the works on "Indian" medicine—astringent, anthelmintic, bitter, stomachic, anodyne, anti-rheumatic, antiseptic, sudorific. Its tender fruits are described as having carminative and stomachic properties and the seeds are said to have a purgative action.

Oroxylin is a flavon. It was first isolated by Naylor & Dyer (1901). Later (1934-36) C. R. Mehta, our chemist isolated it again in a pure crystalline form and showed it to be 5-7 dihydroxy-6-methoxy flavon. The yield of the substance from the root bark is very poor, 0.6%. It is insoluble in water, soluble to a very little extent in alcohol. Hence it is very difficult to use it in experiments, only its suspensions had to be used.

It has little local action. It is not astringent. It has little action on the lower organism. Micro-organisms like B. coli are not killed in its presence nor is their growth affected when the substance is added to the culture medium. So it is neither bacteriostatic nor bacteriocidal when tested on B. coli. The healing of wounds is not affected by it.

It is not toxic when given orally to rats weighing 120 grams in 5 grains doses. No symptoms were observed in rats even after prolonged administration of smaller doses for months.

Tested by Sollmann's method on the earth-worms it was not found to have any action. It slightly depresses the gastroenemius of frog when the isolated muscle is kept in its suspension in saline. So also isolated pieces of frog's and mammalian intestine stop their spontaneous movements in its presence, but recover on washing out the drug. The passage of intestinal contents was also slightly slowed when the drug was given to rats by the mouth. The drug is absorbed to some extent from the intestine as its traces can be recovered from the urine, but also from the faeces. Suspension perfused through the isolated frog's heart and frogs' heart in situ, produced little effect except that due to the blocking of blood vessels by the thick emulsion. On intravenous injection of the emulsion into dogs, no change was seen on the blood pressure, pulse rate or respirations of the anaesthetised animals. In short, the drug has got very little action except a slight depressant action on the muscular tissue (plain and voluntary) which accounts for the slowing of the passage of the intestinal contents. As the drug was found to have no other action for which it could be employed in therapeutics, the investigation was discontinued.

57. Pharmacological enquiry by Dr. B. B. Dikshit at the Haffkine Institute, Bombay.

Plasmoquine.

(*a*) Researches on the action of plasmoquine in pregnancy were continued to see the action on the foetus, when the drug is administered to the mother.

Doses of plasmoquine, equivalent to therapeutic doses in man, were administered to female guinea-pigs in various stages of pregnancy.

The litter given by these animals were observed for two months and it was found that their growth and health did not differ from the normals. In animals therefore small doses of plasmoquine given to the mother during pregnancy do not appear to produce any bad effects on the offspring.

This suggested that the placenta may filter out plasmoquine and prevent it from reaching the fœtus. This possibility was therefore investigated by experiments in animals by employing physiological and chemical tests. The heart and respiratory centre of animals are very susceptible to the action of plasmoquine and records of the activity of these were employed for physiological tests. A large dose of plasmoquine was administered to anaesthetised pregnant animals intravenously and the heart movements or diaphragm movements of the fœtus registered graphically, the fœtus all the time retaining its maternal connections. It was observed that even a large dose of plasmoquine given intravenously to the mother did not in any way modify the action of the fœtal heart or the contractions of the fœtal diaphragm. The heart and respiratory centre of fœtuses are very susceptible to the action of plasmoquine and the fact that these were not affected in any way even when high concentrations of the drug were present in mother's blood showed that sufficient quantities of the drug did not reach the fœtal circulation. A further proof was thus obtained to show that the placenta acted like a barrier and prevented plasmoquine from reaching the fœtus in significant quantities.

Chemical investigations on the fœtal blood and fœtal organs however showed that the placenta does not act as a complete barrier, for small amounts of plasmoquine could pass through the placenta into the fœtus. Experiments similar to those described above were made and it was found that when the fœtal organs and fœtal blood were extracted with the method described last year and tested for plasmoquine, the presence of the drug could be demonstrated in them. Quantitative estimations of the actual amounts of plasmoquine present in the fœtus however revealed that the amount was too small to influence the fœtus deleteriously. These experiments were concerned with large doses of plasmoquine. When therapeutic doses were administered to the mother, no plasmoquine could be detected in the fœtus. These experiments therefore indicate that administration of plasmoquine to the mother in therapeutic doses would probably produce no harm to the fœtus.

(*b*) Experiments were made to study the fate of plasmoquine in the body. It appears that the drug is either destroyed or changed into some other compound fairly rapidly when injected in animals. The nature of this destruction is under investigation and some experiments suggest that some enzymes are responsible for it. The liver and the brain appear to destroy the drug fairly rapidly and acetone extracts of these organs retain the activity.

Atebrin.

(*a*) Investigations on atebrin were carried on lines similar to those described under plasmoquine. By injecting large doses of atebrin to the mother it could be demonstrated that the dye was partially filtered out by the placenta. It appears however that atebrin can pass through the placental barrier more easily than plasmoquine.

(*b*) The action of atebrin on uterine movements of non-pregnant rabbits was investigated by employing Reynold's method which allows graphic registration of the movements of the uterus without giving any

anaesthesia to the animal. It was found that therapeutic doses of atebrin influenced the movements of the uterus very little.

BENZO NICOTINE.

Dr. B. K. Nandi had prepared a quinoline derivative in Manchester called "Benzo-nicotine" with a view to seeing if it could form a basis for further synthesis of anti-malarial drugs. The pharmacological action of this compound was investigated. It was found to possess properties very similar to those of nicotine. Qualitatively the actions are identical with those of nicotine but quantitatively the drug appears to be about 1/3rd as active. The drug is very interesting from purely physiological point of view, but its utility as a basis for synthesis of anti-malarial drugs appears doubtful, unless synthetics prepared from it lose the nicotine like action.

58. Algæ enquiry under the Director, King Institute, Guindy.

The enquiry was started in April 1938 with the object of studying (1) the seasonal changes occurring in the algal flora of water supplies and their relationship to the chemical constituents of the water, and the meteorological conditions of the locality and (2) the influence of algæ growths on modern methods of water purification.

The places for investigation were chosen with a view to obtaining representative samples from the four chief types of water supply sources in the province viz., (1) impounded reservoirs on the plains (2) upland surface water (3) rivers and (4) ground waters (well etc.). The Red Hills Lake near Madras from which the capital city derives its water supply, the reservoirs at Ootacamund situated on the Nilgiri Hills at an elevation of about 7,000 feet above sea level, the water of the Godavari river at Rajahmundry and the wells at Dindigul represent the four types in the order mentioned.

Periodic visits were made to these places. The phytoplankton was collected with the aid of a plankton net, examined in the field and then preserved immediately for further detailed examination. Samples of water were also collected at the same time and their pH, dissolved O2 and CO2 contents were determined on the spot. A detailed analysis was then made of each sample to determine the following : (1) total hardness, (2) total solids, (3) permanent hardness, (4) bicarbonates, (5) carbonates, (6) chlorides, (7) nitrites, (8) nitrates, (9) saline ammonia, (10) albuminoid ammonia, (11) oxygen absorbed, (12) calcium, (13) sodium and potassium, (14) magnesium, (15) iron, (16) silica, (17) sulphates and (18) phosphates.

A detailed examination was also made for identifying the specimens of algæ and determining their relative numerical preponderance.

RED HILLS LAKE, MADRAS.

In April 1938, the Red Hills Lake water was rich in *Peridinium* (a dinoflagellate) and *Phormidium* (a blue green algæ) the latter being present in sufficiently large numbers to impart a light greenish colour to the water. Towards the end of June and in the beginning of July, *Peridinium* decreased rapidly, and by the first week of August it had almost completely disappeared from the plankton flora of the lake. *Phormidium* continued, however, to be present throughout this period in fair abundance. In the first week of August, chlorococcales (green algæ) made their first appearance in the

plankton. Of these, *Kirchneriella lunaris*, *Dimorphococcus lunatus*, *Oocystes Pediastrum duplex*, *Pediastrum simplex*, *Coelastrum microporium*, *Ankistrodesmus falcatus*, and *Scenedesmus quadricauda* were the most predominant forms. Along with these green algæ, blue-green forms such as *Microcystis flos-aquae*, *Meris mopedia*, *Phormidium* and *Aphanotheceae* were also present in comparatively small quantities. Towards the end of August, *Microcystis flos-aquæ* began to increase rapidly in number till it became the dominant form found in the plankton collections and has continued to remain so up to the present.

Phormidium which was consistently present during this period showed a fluctuation in numbers with a sharp rise in the first weeks of August and September and again in the middle of October and November as also in the end of November, and a noticeable fall after each rise.

From the results of the chemical analysis, it would appear that the growth and multiplication of *Phormidium* have a definite relation to the albuminoid ammonia content of the water, high values being found to favour multiplication.

Rajahmundry.

Samples of water and algæ were obtained regularly every month from the Godavari river, from the coagulating tanks and from the filtered water supplied to Rajahmundry.

The river water was always found to contain a relatively small growth of plankton algæ, and to be devoid altogether of plankton during heavy flood. This is due to the fact that a fast flowing habitat is rather ill-suited to the growth and development of true plankters. The river water which is ordinarily heavily silt-laden, contains large amounts of available silicon which would support a plankton composed mainly of diatoms. Thus, the diatom flora which was present in fairly large numbers in June and July showed a marked decrease towards the end of July when, owing to a great dilution of the river water by recent flood, the silica content underwent a reduction of 42%. Stray *Pediastrum* and *Oscillatoria* were also present along with the diatoms in June and July. In August and September, *Oscillatoria* was absent but filaments of *Spirogyra* took its place.

The settling tanks harboured, however, a good algæ growth consisting mainly of the attached species. Alum coagulation followed by sedimentation would appear to remove most of the true pelagic plankton forms from the water, but genera like *Oscillatoria*, some of the filamentous diatoms and *Spirogyra* find a favourable habitat for growth attached to the walls of the sedimentation basins. In June and July, the sides of these basins were covered with a thick felt-like growth of *Oscillatoria* and between August and December, there was a rich growth of *Spirogyra*, which had almost entirely replaced the oscillatorians.

Ootacamund.

Samples were collected from the reservoirs at Ootacamund in June, September and November. There are five reservoirs which supply drinking water to Ooty, viz., the Marlimund Lake, Kodapamund reservoirs, Dodabetta Upper, the Tiger Hills reservoir and the Dodabetta Lower reservoirs. The last three reservoirs form a group situated at a higher elevation than the others. The plankton of the five reservoirs showed a remarkable diversity in composition and in the relative abundance of the forms present. The earthen sides of these reservoirs are rather steep, and consequently bear very little submerged vegetation.

The Marlimund Lake, was rich in *Peridinium* in June. *Desmids* like *Staurästrum* and *Anthidium* were also present in fair number. By September, however, the *Peridinium* had decreased considerably, and the plankton was composed mainly of a species of *Staurastrum*, along with large quantities of *Botryococcus braunii*. Between June and September, the water showed a 50 per cent. increase in its chloride content, a factor that favours the growth of desmids. In November, the plankton of the Marlimund Lake had changed again being then composed of a very rich growth of a desmid, *Cylindrocystis* with comparatively fewer numbers of *Botryococcus braunii* and *Microcystis flos-aquae*.

The Dodabetta Upper, Lower and the Tiger Hill reservoirs supported in June a plankton composed almost exclusively of the filamentous diatom *Melosira*. In September, after the rains, when the level of water had risen considerably in these reservoirs, the Tiger Hill plankton showed a remarkable change being now composed mainly of *Peridinium*, and continued to remain so even in November. This change would appear to be related to the increase in the calcium, silica and iron content of the water between June and November to the extent of 385%, 50% and 100% respectively.

DINDIGUL.

The algal flora of three deep wells supplying the town were investigated and found to differ widely in variety and number.

Collections were obtained from the Nallacheruvu reservoir. *Microcystis* and *Hydrodictyon*, floating forms of algæ constituted the entire flora. They are very resistant to copper used as algicide and offer particular difficulties to the water-works operator. This aspect of the problem is, therefore, being kept in view.

The more important species of algæ isolated from the water in pure culture are being studied with a view to estimating their reactions to different algicides.

Various liquid media have been tried and Knopp's and Moore's solutions with and without the addition of soil infusions were found to yield good results.

The different species of algæ collected during the investigation have been carefully identified and accurate drawings have been made with a camera lucida.

Mortality statistics have been collected to the extent available from the municipalities concerned, and will be carefully studied with a view to seeing if any correlation could be obtained between the predominance of particular type or types and the incidence of bowel diseases.

59. Sandfly fever enquiry under the Director, King Institute, Guindy.

The field work in connection with this enquiry was carried out in Peshawar by Capt. W. M. E. Anderson, R.A.M.C. The laboratory work was carried out partly in Peshawar and partly in the King Institute, Guindy.

FIELD WORK.

This was commenced in Peshawar on April 1. Sandflies were found to be present in fair numbers, and the first case was admitted to hospital on April 10. Surveys were carried out in various barrack areas where the incidence of the fever was uneven, and in no case was there any real

and direct relationship between the number of sandflies in a building and the number of cases of fever occurring therein. In one British unit, thus affected, the important factor appeared to be the presence of a derelict building, whose walls were in a dilapidated state and lying closely adjacent to the barrack building in which most of the cases were occurring. A thorough renovation of this empty building produced a satisfactory reduction in the number of cases. In several Indian Troops lines, unequal incidences were investigated, and the chief cause appeared to be geographical i.e. that the places most heavily affected were those populated by men who came from outside the "sandfly areas" and particularly was this among hillmen such as Dogras and Kumaonis.

There is a commonly held view, which is propounded by such authorities as Chandler, that sandflies do not fly more than a few feet above ground and that persons sleeping on the upper storey of a building enjoy protection thereby. Work was carried out in some double storied barracks to determine whether this statement could be endorsed or otherwise. It was found that sandflies were present in almost equal numbers on both floors and that the incidence of the fever was, if anything, higher among the persons sleeping upstairs. As a final test, a light trap of simple type was placed 70 feet above ground on each of three nights and six *P. papatassii* were caught.

Experiments were performed to determine the minimum wind speed against which a sandfly could not fly, and the results indicated that the figure is in the region of 1.6 to 1.7 miles per hour. This is the speed of an ordinary non-oscillating table fan, running at its slowest speed at a distance of 6 to 7 feet. It is to be emphasised that this work was carried out under conditions somewhat different to those in nature, i.e., the work was done in the day-time, when the sandflies are possibly more sluggish than at night, and secondly, no bait was provided to stimulate the flies to extra efforts. These two facts should be taken into consideration in the practical application of the above stated results.

Soil disinfestation work was carried out at Nowshera to see if the number of flies in a building could be materially reduced by large scale larvicidal measures. A solution with larvicidal powers had to be obtained and it was decided to use a saturated solution of naphthalene in kerosene, both of which substances are cheap and easily obtainable. A 15% solution can be obtained after standing for several days, but even after a few hours a solution of 10% to 12% is available. An isolated building was selected for the work and after a preliminary survey by a squad of sandfly collectors all likely breeding places within a radius of 120 yards were treated with the solution by pouring it on liberally. This distance allows a liberal margin over the theoretical range of flight of a sandfly (50-80 yards). This treatment of the soil was repeated after six weeks and weekly surveys were made by the collectors, both of this "test" area and also of a control area. The number of flies caught in the "test" area showed no appreciable decrease, and consequently the work must be regarded as not successful. This experiment, however, drew attention to the heavy financial effect of this type of work, and it is computed that even if the cost of the solution be as low as Re. 1 per gallon, the cost of a single treatment of the Peshawar Cantonment area would be approximately a quarter of a lakh of rupees. That the solution had definite larvicidal properties was demonstrated by the fact that at the same time as the work in Nowshera was being commenced, artificial breeding places were made in Peshawar and after fourteen days

(i.e. during the larval stage) were treated with this solution, which was applied to two such places by pouring on liberally and to two by spraying. Neither of the treated places yielded any adult flies, but from both of the two control places, they hatched out in large numbers. The most satisfactory preventive method would appear to be the obliteration of all places likely to shelter the developing fly. This requires constant supervision, and a properly trained working party to prevent the accumulation of rubbish dumps, and to close all cracks in the sides of nullahs and the walls of houses. Work on these lines has been carried out intensively at Landi Kotal and Peshawar by the authorities for several years past and has resulted in a marked decrease both in the number of sandflies and also in number of cases of the fever.

Forty samples of earth have been examined for the presence of sandfly larvæ of which two have been positive. The usual technique has been employed, namely, straining the earth through a series of metal sieves of gradually diminishing mesh and final flotation on a concentrated sugar solution. No fresh facts concerning breeding places have been brought to light. A new apparatus, which carries out this process automatically, has just been received and by means of this it is hoped to extend the scope of the work.

Towards the end of October, the Peshawar section of the enquiry was permitted to carry out some experiments in spraying rooms with a .5% "lethane", solution, by means of a "Phantomyst" atomiser, the property of Peshawar District Headquarters. This work was curtailed by the end of the sandfly season, but the results indicated that this solution is definitely insecticidal. It is hoped that this apparatus may be available for further work and that various other solutions such as "Pyrocide 20" and "Desquito" may be tested.

Laboratory work.

To date 180 specimens of serum have been collected from sandfly fever patients, during both the febrile and convalescent stages. After dessication these are being despatched to the King Institute, Guindy, for further study.

It was noted by the egg inoculation technique that the virus was present in the blood regularly for 7 days. In a few cases its persistence has been noted up to a period of 5 weeks. The virus is now at its 67th passage on the chorio-allantoic membrane. In flask cultures it has been maintained through 51 serial passages. Attempts to increase the virulence of the virus to mice by serial intra-peritoneal and intra-cerebral passages, have failed entirely. The virus was also non-pathogenic to monkeys (m. sinicus). It was found to be circulating in the blood of monkeys up to 11 days after inoculation in majority of cases and up to 19th day in one monkey. Neutralising antibodies have been demonstrated in these sera for at least 6 months. Since the cultured virus was found to be non-pathogenic to laboratory animals, it was decided to use it as a vaccine in human volunteers to determine its value as a prophylactic against sandfly fever. The observations were carried out in Kasauli with the co-operation of Capt. W. M. E. Anderson, R.A.M.C., and Dr. R. O. A. Smith. Sixteen volunteers were chosen for the experiment. Eight volunteers received the vaccine in 2 doses, 1 c.c. and 1.75 c.c. for each dose at an interval of one week. The other 8 were treated as controls and received normal saline instead. No rise of temperature or any untoward reactions were observed in any of the volunteers so inoculated.

The sera of the 8 test volunteers were tested 5 days after the first dose for the presence of virus and the virus was demonstrated in five of them. Four out of the seven sera tested 35 days after the second dose of the vaccine showed the presence of the neutralising antibodies. These results afford evidence regarding the potency of vaccine used. Finally both the control and test volunteers were inoculated with pooled sera from cases of sandfly fever which has been previously tested for the presence of virus. The results were as follows :—out of 6 test volunteers three escaped infection, two had a mild attack and one a typical attack. Out of the 7 in the control group two had typical attacks and five escaped the infection. The results were thus inconclusive. However the attacks in the test group were amongst those in whom either the virus or neutralising antibodies had not been demonstrated. The attacks in the control group were much more severe than those in the test group.

60. Endemic fluorosis enquiry under the Director, King Institute, Guindy.

This enquiry was started in April 1938. A general survey of parts of the Nellore and Bellary districts has been carried out and the incidence of mottled enamel in children and of bone affections in adults has been recorded. Samples of water from the surveyed area in the Nellore district have been examined for their fluoride content, with a view to correlating the incidence of fluorosis with the concentration of fluorides in water. 65 per cent. of the samples showed a fluoride content of 1 p.p.m. and over ; 37 per cent had a fluoride content of 2 p.p.m. and over. The latter correlated very well with the incidence of the severe manifestations in teeth and bones. Staple articles of food and greens grown in the severely affected area of the Nellore district have been collected, dried in air, and stored for estimating their fluoride content. The methods of estimation in vogue are all being tried in order to determine the most suitable consistent with ease and accuracy. The Willard and Winter distillation method has not given uniformly satisfactory results and so the methods outlined by (*a*) Scott and Henne and (*b*) Penfield are being tried.

A general survey in Bellary district was carried out in seventeen relief camps to which people from the five eastern taluks of the district were drawn as the result of the prevailing famine conditions in the area. Forty-eight thousand people (men, women, and children) out of a total of nearly sixty thousand, were included in the survey. Mottled enamel was observed in 70 per cent. of children of the age groups 0 to 16 years ; bone affections were not, however, severe, though mild to moderate lesions were clinically detectable in about 50 per cent. of the adults examined. Samples of water from the villages from which these people hailed to the camps were requisitioned from the District Health Officer, for estimating their fluorine content. 31 samples of water from 24 villages known to be inhabited by people showing symptoms of dental fluorosis (on the basis of the survey of famine camps) have since been received and found to contain fluorides fanging from 0.5 to 2.4 p.p.m. Of these, 24 samples contain less than 1 p.p.m. The high incidence of fluorosis in these villages is, therefore, a matter requiring further elucidation.

A detailed house to house survey was carried out in Podili town and its hamlets and also in a portion of Darsi town supplied exclusively by a well constructed by the District Board in 1935, its fluorine content being

3 p.p.m. A detailed examination was made of 2,300 people (men, women, and children) with particular reference to mottling of teeth in children, and to affections of bones, of spine, chest and extremities in adults. Their dietetic and other habits, sources of water supply and all other special factors operating adversely were carefully recorded. The symptoms noticed with regard to teeth and bone affections, were classified in each case as mild, moderate and severe. Cases showing vague symptoms or early bone lesions in the extremities have been ignored in the final analysis. The analysis of figures for the whole area surveyed revealed the following features :—

(1) 74 per cent. of children showed mottling of enamel ; 23 per cent. mild, 20 per cent. moderate and 31 per cent. severe.

(2) Of 1,115 children thus examined, 588 were boys and 527 were girls and the incidence of mottling was almost the same in both sexes, the percentage being 74.2 for boys 75.2 for girls.

(3) The severe type of mottling was, however, observed in 34.3 per cent. of boys and in only 27.9 per cent. of girls examined.

(4) 141 out of 387 children of the age group of 0 to 5 years showed a mild type of mottling. The incidence of this condition affecting the milk teeth, was 36 per cent. in boys and 36.9 per cent. in girls. The findings of American observers would, however, appear to show that only the permanent teeth are usually thus affected, while the milk teeth are generally exempt from the effect of flourine intoxication.

(5) The toxic level of fluorine in water at which mottling of the teeth occurs appears to be in the neighbourhood of 1 p.p.m.

(6) Of 1,192 adults, whose case histories have been scrutinized, 601 were men and 591 were women of the ages of 21 to 50 and above. The presence of bone lesions in one or more of the following parts of the body viz., spine and chest, was used for classification into mild, moderate and severe. 887 adults or 74.4 per cent. of the total number examined showed bone affections, the sex incidence being 75.2 per cent. in men and 73.6 per cent. in women. The mild cases were represented by 39.6 per cent. of men and 37.2 per cent. of women, while the corresponding figures for moderate and severe types were 22.8, 21.1, and 13.0 and 15.2 per cent. respectively.

(7) Malapalli, a hamlet of Podili town, occupied exclusively by Malas (sweepers) showed by comparison a much higher incidence of generalised bone affection, the severe types being found in 28.6 per cent. of men and 30.0 per cent. of women. The corresponding figures for Podili town area were 12.0 per cent. and 12.9 per cent. respectively. The water supply to this hamlet was exclusively from a well with a fluorine content of 3 p.p.m. and the Malas of this hamlet are not given access to the general drinking water well (fluorine content 1.2 p.p.m.) used by the Caste Hindus and Muslims. Apart from the habits of the people, their diet etc., the significance of which remains to be investigated, Podili town proper and Malapalli provide a significant comparison of the effect of concentrations of fluorine in water on the severity of the disease.

(8) Unlike the hamlets of Podili, it was possible to distribute the population of Podili town proper amongst the different occupations. From the data classified on this basis, it was found that men engaged in pursuits involving light manual labour (clerks, teachers, merchants, artisans etc.,) showed a smaller incidence of bone lesions and severe manifestations were

found in only 9 per cent. of those examined (15 out of 179) as compared with the agricultural and other labourers doing hard manual work who showed a 16.2 per cent. incidence of severe affections (25 out of 154).

(9) Judged by the length of residence, the incidence of bone affections appeared to be higher in adults who had resided for over 15 years than in those who had resided for less than 15 years in the area. This was true of both sexes.

Laboratory experiments have also been carried out on the removal of fluorides from water, using various chemicals, absorbents and base exchange materials, such as alum, aluminium powder, aluminium and copper zeolites, tricalcium phosphates, activated carbon and defluorite, some of which were specially prepared for us in England by a firm of water engineers. Tricalcium phosphate and defluorite alone appear to be the most promising in this respect and further work is in progress.

61. Snake venom enquiry by Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

(1) Enzymes in snake venom.

The presence of oxidase, proteinase, esterase and peptidases in the venoms of cobra (Naja Naja) vipera russellii, echis carinata and bungarus fasciatus, has been detected. The determination of the critical inactivation temperature of the proteinase, the pH at which its activity is maximum and the effect of poisons like HCN on its activity, indicate that the proteinase is very probably trypsin. The peptidases present in the venoms mentioned above, can only hydrolyse the laevo rotatory forms of the di- and tri-peptides and not the corresponding dextro rotatory forms. Two papers containing the results of these investigations have been communicated for publication.

(2) Isolation of the toxic constituents of snake venom

(*a*) *Cobra venom.* The haemolysin and the neurotoxin present in cobra venom have been separated from each other by taking advantage of their difference in solubility in the presence of electrolytes such as sodium sulphate or sodium chloride. A solution of cobra venom is subjected to fractional precipitation in three stages using increasingly higher concentration of sodium sulphate at each successive stage; the major portion of the neurotoxin is found in the final filtrate and the whole of the haemolysin together with some neurotoxin in the precipitates. The neurotoxin is removed from the filtrate by adsorption on and elution from the surface of tungstic acid. It is further purified by fractional precipitation with methyl alcohol at 4° C. 1 mg. of this purified sample contained 110 M.L.D., whereas 1 mg. of crude cobra venom obtained from Haffkine Institute contained only 3.3 M.L.D. Therefore the purified neurotoxin is thirty times more active than the crude venom. This purified neurotoxin does not contain enzymes such as choline esterase, proteinase, etc. The precipitates containing haemolysin are dissolved in water and the haemolysin separated by fractional precipitation at 22 per cent. sodium chloride concentration. The crude haemolysin thus obtained was further purified by removing part of the admixed proteins by heat coagulation, followed by fractional precipitation with metaphosphoric acid. Weight for weight the purified haemolysin fraction is seven times more active than the crude venom. A paper containing these results has been published.

(*b*) *Russell's viper venom.* By fractional precipitation of a solution of Russell's viper venom by sodium sulphate followed by absorption on and elution from the surface of aluminium hydroxide C (prepared by Willstatter's method) it has been possible to concentrate the toxic substances in a fraction which contains only 13 per cent. of the protein of the crude venom. A paper containing these results has recently been communicated for publication to the Indian Journal of Medical Research.

(3) ACTION OF REDUCING AGENTS ON SNAKE VENOM.

The action of a number of reducing agents such as sodium bisulphite, hydrogen sulphide, ascorbic acid, cysteine etc., on Russell's viper venom has been tried and it has been found that they all destroy the major portion of the neurotoxin in the course of a few hours. A dilute solution of sodium bisulphite is found to destroy about 90-95 per cent. of the neurotoxin without appreciably affecting the haemorrhagin and the blood coagulating principle. This suggests a possible method of separating the two latter substances from the neurotoxin.

(4) DIFFUSION AND CATAPHORESIS OF SNAKE VENOM.

The cataphoretic behaviour of the venom of vipera russellii has been investigated within the range pH 2.0-9.0.

The neurotoxin of Russell's viper venom has been found to have its isoelectric point between pH 5.8 to 6.0. It is thus amphoteric in nature. Diffusion experiments also show that the molecules of the neurotoxin of Russell's viper venom are much larger compared to that of cobra (Naja Naja) venom.

62. Investigation into the aetiology of infantile biliary cirrhosis with special reference to anaemia by K. Narayana Murthi at the Research Department of Pathology, Medical College, Madras.

CLINICAL.

Twenty-one infants between the ages of 6 months to 2 1/2 years with hepatic and splenic enlargements were examined. History of the incidence of the disease in the family, the diet, and the occurrence of successive symptoms during the progressive course of the disease are noted. As there is no single test for differentiating the disease from many other diseases in the tropics, causing enlargement of liver and spleen in children, the following tests are carried out systematically.

Blood tests: 1. Cytological, 2. Van den Bergh, 3. Kahn and Wassermann, 4. Haemoclassic test, 5. Frigility of R. B. C. and 6. Halometer readings.

CYTOLOGY.

All the infants showed marked anaemia, the haemoglobin percentage varying from 20 to 60. Normoblasts, megalo-blasts, and megalo-cytes and large preponderance of polymorphs were present in 8 infants. No malarial parasites or K. A. bodies were found in any of them. Fragility of the R. B. C. was normal. Halometer readings were 7.4 to 6.4. The blood picture was suggestive of a severe grade of secondary anaemia probably nutritional in origin. A deficiency of vitamins in the diet may play

a part in the etiology of the disease. The increase of polymorphs, a very high cellular count in the ascitic fluid and the common occurrence of a low persistent temperature are suggestive of some infection which is being investigated.

Bacteriology.

Microscopical examination of the stool revealed no ova or cysts, but the presence of fat globules was common.

Culture of stool and urine was done in every case but no pathogenic organisms were discovered. The commonest organisms found were B. coli, enterococcus, B. asiaticus, B. paracolon group, and a few spore bearing bacilli.

Attempts were made to determine whether these organisms, which are non-pathogenic ordinarily to children, attain pathogenic tendencies when cultured on media made with agar, liver and spleen.

Sub-cultures of these organisms are being maintained in order to contaminate the food given to the experimental animals, in which gastric, duodenal and intestinal fistulae have been made. Also, the food is contaminated directly from stool and urine of the infant suffering from the disease. Work in this direction is proposed to be carried out to study the lesions, if any, developed in the liver in experimental animals.

Vegetative nervous system.

The great incidence of the disease in children of a particular class and social status (Brahmins) suggest the possibility of the disease, having its origin in the disturbance of the vegetative nervous mechanism. Whether any disturbance of the autonomic nervous system may be the determining factor in the causation of the symptoms of the disease is in question. Experiments are in progress to put out of action either of the branches of the vegetative nervous system and to note the changes, if any, macroscopic or microscopic in the liver and spleen of experimental animals.

Therapeutics.

To gain further knowledge about the relationship of the vegetative nervous system to the disease, selected patients were treated with insulin and glucose, or acetyl-choline, or zinc salts etc. Work in this direction gives much hope in the elucidation not only of the etiology but also in the treatment of the disease.

Virus.

In a typical case of infantile cirrhosis 5 c.c. of blood was taken, defibrinated and the allantoid membrane of 7 growing chicks inoculated. It is of interest to note that 3 gave a positive reaction, on the membrane, 3 negative, and one deteriorated. The allantoid membrane, both normal and abnormal is submitted for section-cutting. The sections are stained by Giemsa overnight, with a view to find, if there were any inclusion bodies. Work in this direction is progressing.

Castor oil and its effects on experimental animals.

There is a strong presumption that castor oil plays some part in the causation of the disease. Animals used are (1) albino rats, (2) guinea-pigs and (3) monkeys. Rats and guinea-pigs are given orally 2 c.c. to 3 c.c. of castor oil every day. A monkey, 6 months old is given half an oz. of the oil on alternate days. Controls are maintained. The monkey, since starting the experiment has lost 80 grms. in weight.

Chemical analysis.

Different brands of castor oil were chemically analysed to find out the ricinoleic-acid content, which is the purgative principle of the oil. Pure oil was separated by a special process and the sodium soap of it was injected into the rats daily to ascertain the toxic changes it produced in the liver. An elaborate technique was devised to determine the sodium ricinoleate in the blood from the portal vein of the experimental animal, to prove absorption and toxicity on the liver cells.

The effect of sodium-ricinoleate on the intestinal flora and fauna was also studied with a view to observing its bearing on the intestinal flora of the infants, who are habitually given castor oil.

Different castor oil seeds have been found to have different ricinoleic acid content. Therefore, the oils sold in the market have varying purgative value.

Ricin.

It is a powerful toxic substance, isolated in a pure state, from the endosperm of castor seeds and injected into frogs at varying temperature, of 50°C., 80°C., and 100 C°., through different routes, *viz.*,(1) subcutaneously (2) intramuscularly, (3) intra-peritonealy. All the frogs died at varying periods of time, except one which had the ricin boiled and injected at 100°C. It is of interest to note that all the frogs extruded a large number of nematodes which are probably the normal inhabitants of the intestine and the ricin acting as a powerful helmenthetic. Ricin, taken from 3 castor seeds, was reported to kill an adult human being. Cushny opines that 1/1000 mgm. per kilo body weight, is enough to kill an adult human being. Three castor seeds were taken, pure ricin was isolated and injected subcutaneously, into a frog, without any effect. The question of toxic dose awaits elucidation. The identification of ricin by biological methods, as described by Cushny, was not successful in this laboratory.

Injections of ricin in non-lethal doses is being carried, on albino rats to find, if any, the lesions in the liver.

X-ray examination.

X-ray photographs of long bones and the pituitary fossa were found to be normal. Pituitary fossa was visualised with the idea of finding out the possible connection of the pituitary gland with the disturbance of the fat and carbo-hydrate metabolism in the infant. So far three cases have been X-rayed and further results are awaited.

Autopsy.

Post-mortem examination was done on two cases of infantile cirrhosis and the histo-pathology of the internal organs was studied. No inclusion bodies could be made out from the specimens.

Pathological specimens : Two specimens of cirrhosis of the liver (a) an infant (b) an adult (for comparison) were submitted for investigation. Infant's liver was injected with lipiodal through the inferior vena cava, and X-rayed. The portal veins and their capillaries were well visualised, thereby showing no obstruction.

Into the adult liver a substance opaque to X-rays is made in the laboratory and injected through the hepatic vein with a view to bringing into prominence the hepatic tree and portal tree. This work will be followed when post-mortem liver specimens become available.

63. Researches on Flies by Dr. C. Strickland at the School of Tropical Medicine, Calcutta.

Attention has been mainly confined to one of the most important matters in the control of domestic flies, namely their natural and seasonal breeding habits in Calcutta and its immediate outskirts. The total number of *Calyptrate Muscoidea* (excluding Anthomyidae) bred out has been 11,881 which have been identified, and they belonged to the following genera :— *Musca* (9 species), *Orthelia* (1), *Chrysomyia* (2), *Sarcophaga* (4), *Stomoxys* (1).

Apiochaeta spp. among the *Acalyptrates*, which sometimes parasitises man has also been bred out.

REPORTS OF ADVISORY COMMITTEES.

REPORT OF THE CHOLERA ADVISORY COMMITTEE.

The Committee met at 2-30 p.m. on December 14th 1938, and considered the reports of the several enquiries and submit the following summary of the work done and the findings for information :—

I. ENQUIRY INTO THE CHOLERA VIBRIOS AND RELATED VIBRIOS UNDER THE DIRECTOR, CENTRAL RESEARCH INSTITUTE, KASAULI.

(*a*) *Serological diagnosis of V.cholerae.*

The work on this subject which has involved the investigation of (*i*) the value of tests with our O serum (*ii*) the incidence and significance of other O types (*iii*) the incidence and significance of H agglutinators has been completed and the results of the work summarised in a report to the Office International d'Hygiene Publique. The conclusions arrived at have been accepted by that body and have been embodied in the report of the Cholera Commission.

(*b*) *Differential isolation of the vibrios.*

The widespread distribution of vibrios which has been shown to exist in India makes it necessary to employ methods of culture which will favour the chance of isolation of *V.cholerae* if accurate information as to the distribution of this organism in water and other sources is to be obtained. Improved methods for the purpose are necessary for field investigations and the enquiry has mainly been engaged in studying this subject during the year.

A large number of procedures has been tried and a method has finally been involved which under laboratory conditions has been found very effective. This consists in a modification of the Wilson & Blair bismuth sulphite enrichment medium. Alterations in proportions of some of the ingredients have been made and mannose substituted for mannite. The medium suppresses organisms of the *coliaerogenes* group and Proteus and permits of growth of very small inocula of vibrios. It gives differential growth of mannose fermenting vibrios (including *V.cholerae*) against mannose non-fermenting strains. Under certain circumstances it shows a considerable advantage over the ordinary peptone water medium and recent alterations in the medium to improve on the results obtained in a first field trial have increased the value of the method. The difficulty still exists in obtaining full differential isolation of *V.cholerae* that many vibrios other than *V.cholerae* found in water and in the stools of healthy persons also ferment mannose and would not be separated from the cholera vibrios by means of the medium.

(*c*) *Studies on fermentable carbohydrates.*

Investigations have been carried out for the purpose of obtaining if possible a substance specifically fermentable by *V. choleræ* and not by other vibrios. The line followed has chiefly been by synthesis from mannonic acid. A large number of substance have been produced and their structure in relation to fermentation studied. None of the preparations has so far been found to be differentially fermented by *V. choleræ*.

The chemical side of the enquiry has also been directed towards the study of factors in water favourable to the survival and growth of *V. cholerae* both in natural and synthetic waters and a basis of information has been obtained which can be applied to field investigations.

2. CHOLERA ENQUIRIES UNDER THE DIRECTOR, KING INSTITUTE, GUINDY.

1. *Observations on the distribution of the types of V. cholerae in the Madras Presidency.*

These were continued during the year. Of 931 strains examined, 613 were derived from Madras, 232 from the Northern Circars and Ceded Districts and 86 from the Southern Districts. All the strains derived from Madras, the Northern Circars and Ceded Districts were of Inaba sub-type consistent with the observations of the previous years. The strains from the southern districts isolated earlier in the year were of Ogawa sub-type ; but in the later part of the year, when there were only sporadic cases or small village outbreaks, they were Inaba. It is interesting to note that this change in type occurred during a period of comparatively low incidence. It is proposed to carry out during the next year more intensive observations in the Cauvery Delta with a view to determining the conditions under which this change occurs and if the relation between the low incidence and the change in the type of vibrio is only accidental or really significant.

2. *Isolation of vibrios from natural and other sources of water.*

This was continued during the year. 145 samples of water from different sources were examined and vibrios isolated from 100. The 45 negative samples include 39 which had been chlorinated or otherwise treated. None of the vibrios were agglutinable by Group I 'O' serum. A seriological grouping of the Nag vibrios from water and from cases is in progress.

3. *Cholera carrier investigation in Madura.*

This survey has been continued by the Municipal authorities of Madura. The city continued to be free from cholera throughout the year and the figures are taken as an index of the conditions during an inter epidemic period. In an examination of 6,301 residents of 3 divisions, no agglutinable vibrio carrier was detected. Non-agglutinable vibrios were found in 130 specimens.

4. *Cholera fly transmission enquiry.*

As had been stated last year, the object of the enquiry was to study the role of the house-fly in the spread of cholera. Dr. Soparkar has continued his work on this subject and has confirmed his last year's finding *viz.*, that the cholera vibrios when ingested by the common house-fly, at a single feed, were rapidly destroyed in the body of the fly, and that the vibrios could not be isolated except during the first few hours after food. These remarks apply only to the isolation of true cholera vibrios from flies. Dr. Soparkar also confirmed his last year's finding that an extract of flies had a bactericidal action on cholera vibrios. The nature of the principle contained in the extract was investigated in greater detail. It was found that :—

1. The action of the extract could not solely be attributed to the normal products of metabolism present in the fly, *e.g.*, urea, ammonia, etc.
2. When the extract was heated to 115° C. a precipitate of coagulable protein appeared. The bactericidal principle was found

in the clear supernatant fluid but not in the precipitate. It was not an enzyme.

3. The principle is soluble in water, but insoluble in ether and acetone.
4. It was present in the filtrate obtained after precipitation of the extract with phosphotungstic acid.
5. When the crude extract was dialysed, the principle appeared in the dialysato and the residue was inactive.
6. The extract acted quantitatively and what is more important specifically *i.e.*, it had action only on vibrios, but not on other organisms studied.
7. An interesting observation is that if the original extract is filtered through Elford's gradocol membrane the filtrate is found to be only feebly active.

As had been stated, the extract has a rapid bactericidal action depending, of course, on the number of organisms used in the test. If the concentration of the extract is made insufficient for such an action, the few organisms escaping lethal effect were found to multiply. In some cases they were found to be true cholera vibrios. In a few instances, however, changes were observed in the strains which are under further study.

3. CHOLERA BACTERIOLOGICAL ENQUIRY UNDER MAJOR C. L. PASRICHA AT THE SCHOOL OF TROPICAL MEDICINE, CALCUTTA.

Isolation from cases of cholera.

In the first six months of 1938 from 631 cases 58% showed the presence of agglutinating vibrios, 7% showed vibrios of other types and 35% no vibrios. In the next 3 months of 148 cases 10.7% yielded agglutinating vibrios and 1.3% other vibrios.

The low figures in the latter period indicate the necessity for improved isolation methods.

In this connection a parallel series, done on 24 stools with a new medium containing haemoglobin showed 16 of these to contain agglutinating vibrios while bile salt agar showed 6 only positive. The above results were obtained by direct plating of the stools.

Agglutinins in the blood.

(a) Cases showing agglutinating vibrios. 113 patients were examined utilising O and H & O suspensions. The results indicate that in the majority of the patients agglutinins are present between the 7th and 10th days. The H agglutinins appear earlier and in higher dilution both for the homologous and standard Inaba strains.

(b) Cases bacteriologically negative—Of the cases examined in section 1, fifteen which had proved bacteriologically negative, were examined for *V. cholerae* agglutinins ; 4 of these showed agglutinins at titres between 1/40 and 1/320 with the boiled suspension and 5 between 1/20 and 1/640 with the un-boiled suspension. It is noteworthy that one case showed "H" agglutinins at 1/320 and no "O" agglutinin in dilution of 1 in 10.

(c) Contacts of cholera patients—Thirteen were examined and had no agglutinins in their blood at 1/10 and upwards.

(d) Healthy persons.—110 of these were examined and showed no agglutinins in their blood at 1/10 and upwards.

Bacteriological types isolated.

(*a*)—From 250 patients studied 34 yielded *V.cholerae* of the Ogawa type. The remainder were Inaba. Fifteen of these yielded both Inaba and Ogawa strains. (*b*) Two non-motile agglutinating vibrios have been isolated. One is of the Ogawa sub-type and other is under study. (*c*) A vibrio isolated from a nearly full term fœtus produced abortion in preghant guinea-pigs and the vibrio was recovered from the guinea-pig fœtus. This is an interesting finding and more work on these lines is in progress.

Vibrios from non-cholera individuals.

575 non-cholera individuals yielded 4.3 % of vibrios of all types, 3 of which were agglutinating strains.

Vibrios in non-human sources.

Inagglutinating vibrios were isolated from a variety of local animals.

Vibrios from natural waters.

128 samples were examined of which 70% were positive. All strains were inagglutinable. A comparative chemical study of these waters shows that there is no apparent relationship between the nature of the water and the presence of the vibrios.

Cholera vaccine.

A paper published in the Indian Medical Gazette gives details of a test of 14 vaccines bought in the open market. Not all these vaccines gave evidence of being prepared from suitable strains.

Biochemical work.

The blood sedimentation rate in the majority of cases of cholera is increased. Methods have been standardised for the full examination of blood chemistry in cases of cholera and it is hoped to carry out tests on actual cases next year.

Additional work.

In addition a large amount of work was done to determine the H-ion concentration of stools in cholera patients collected at different times; in attempts to isolate the vibrio from the urine and in examining the reaction of the blood of patients during the acute stage of the disease. The morphology of cells and colonies of *V.cholerœ* was studied by the method of impression smears.

The presence of Vi antigen was tested for by the use of acid treated suspensions. This method showed the presence of residual agglutinins after absorption.

Work was done on the viability of vibrios in natural waters. It showed that this is extremely poor especially in hill waters.

An attempt was also made to group non-agglutinating vibrios according to the action of phage upon them. Four groups were defined and it is proposed to complete a correlation of these groups with their serological groupings.

4. CHOLERA CARRIER AND EPIDEMIOLOGICAL ENQUIRY UNDER DR. R. B. LAL, ALL INDIA INSTITUTE OF HYGIENE AND PUBLIC HEALTH.

The object of this enquiry was two-fold, *viz.* (1) to test the efficiency of a differential medium for the isolation of agglutinable vibrio from

natural sources and (2) to apply this technique for studying the distribution of the agglutinable vibrio in human and environmental sources in endemic and epidemic regions. The differential medium to be tested consisted of a modified form of Wilson and Blair's liquid enrichment medium in which broth was replaced by peptone and mannose was substituted for mannite and brilliant green was omitted. The material used to test the medium were samples of stools collected from cholera and non-cholera patients, contacts of cholera cases and healthy persons not giving history of recent cholera.

The differential medium gave better results than the peptone water medium in isolating all types of vibrios from samples of stools but not from water samples. However, there was a distinct advantage with the differential medium so far as isolation of agglutinable vibrios was concerned. With further modification of the medium the results were still better with regard to the isolation of agglutinable vibrios and suppression of non-agglutinable vibrios. A further advantage of the use of the differential medium is that coliform organisms are suppressed and isolation is therefore easier.

In two instances the agglutinable vibrio was found in samples of water from tanks, which were used by the villages for drinking purposes. No cases of cholera occurred either before or after the data of isolation of the vibrios.

The agglutination titre of a number of healthy individuals against the agglutinable vibrio was determined. If a titre of 1 in 25 is arbitrarily taken as positive 68% of 50 persons in one village and 71% of 38 persons in another village were found to react. No cholera deaths had been recorded in either of the villages for a period of two and a half years or more. No difference in the percentage of reactors was found amongst the inoculated and the un-inoculated persons, nor amongst those using the infected tanks for drinking purposes and those not using them. In another village where no cholera deaths had been reported for 25 years sera of only 4 out of 40 persons examined were found positive. These findings would probably suggest that sub-clinical infection with agglutinable vibrio is a common occurrence in these areas even when no recognisable cases of cholera have occurred.

Chemical analysis of water from different sources was also carried out. The results have not yet been properly analysed.

5. Cholera statistical enquiry under Dr. R. B. Lal, All-India Institute of Hygiene and Public Health, Calcutta.

Having formed 19 homogeneous cholera districts in the Southern and South-Western portion of Bengal a series of investigations were undertaken on the epidemiology of the disease taking the district as a unit.

Association of the incidence of cholera mortality over a number of years with each of the four metrological factors *viz.*, mean temperature, mean relative humidity, rainfall and number of rainy days for the corresponding months was investigated. The method used was co-variance which removes the seasonal and yearly effects and measures the degree of correspondence between the deviations in the two varieties in time series. No significant correlation was observed except in one area where temperature and cholera mortality were positively correlated. It is proposed to re-investigate this problem with varying lag periods.

Trend lines were fitted to cholera mortality for the period 1901-32. Decline was observed in all the homogeneous districts but it was significant only in ten instances. The reasons for the occurrence of the differences in the slope up the trend lines require further investigation.

A new classification of the homogeneous cholera districts in respect of their endemicity and epidemicity was made in which the disturbing effects of the size of population and the number of units composing the districts were eliminated. This classification which is more accurate than the one previously obtained will, it is believed, materially help in the conduct of the field investigations.

With a view to obtaining a comprehensive idea of the conditions prevailing in the homogeneous districts of different epidemiological characters, relevant information contained in the District Gazetteers was extracted. These studies have not proved very fruitful.

Rogers, in his method of forecasting cholera epidemics makes use of 0.4 mm. of absolute humidity as the critical level. Four-fold contingency tables were prepared to test the association of rising cholera mortality with absolute humidity above the critical figure. The results obtained did not support this hypothesis.

The new method of forecast described last year was successfully applied to cholera experience in Calcutta during 1936, 1937, and 1938 (in so far as the figures were available). It was further found that his method could be applied to ten of the 19 homogeneous districts but it was not applicable to the rest.

6. CHOLERA ENQUIRY UNDER DR. S. R. PANDIT AT THE PASTEUR INSTITUTE, SHILLONG.

The field unit of the enquiry started work in the Goalpara District in the 1st week of July and upto the middle of November had made 1202 examinations, of which 346 were from the epidemic areas and 856 from a cholera-free area. In the epidemic areas specimens from 46 cases, 27 convalescents, 73 healthy contacts, 20 water sources and 180 healthy individuals were examined, vibrios being isolated in 76, 26, 7 and 2% respectively. The agglutinable vibrio was isolated in 28 out of 46 cases, 1 out of 27 convalescents, 3 out of 73 healthy contacts and none from water. The modified Wilson-Blair method was not employed.

In the cholera-free area, an examination of 177 samples of water and stools of 679 healthy individuals yielded vibrios in 30% and 1.2% respectively.

During the year 322 specimens of non-cholera stools were examined for vibrios. They were routine specimens received for dysentery, enteric and helminthic infections etc. 58 (18%) were positive for vibrios.

This from stools of 85 healthy individuals, the inagglutinable vibrio was isolated 11 times (1.3%), from 46 cases 7 times (15.2%), from 27 convalescents 6 times (22.2%) and from non-cholera stools 58 times (18%). From 197 samples of water the negative vibrios was isolated 61 times (31%). The conclusion seems to be justified that inagglutinable vibrios are natural inhabitants of waters and that such vibrios taken in with water have little chance of survival in the gut of healthy individuals, but in those with an intestine disturbance they are more often met with in their stools. Exclusion of the non-fermenting type of vibrios does not affect the conclusion.

Doubts have arisen regarding the classification of the non-fermenting vibrios. Unlike other types, none of these, of which over 50 have been studied, is agglutinated by the flagellar antibody of cholera vibrio and none is acted on by any of the 12 phago types; the bacillary shape with one or more polar flagella rather than the vibrio shape is almost invariable among these. They appear to have a greater affinity to *Pseudomonas*, whose fermentative property is low, than to the *Vibrio* group.

Cholera outbreak at Manipur.

A short bacteriological investigation during the early part of an outbreak of cholera at Manipur was undertaken in April. Stools of 19 cases were examined but the agglutinable vibrio was not isolated from any. Blood sera of 12 convalescents were put up for cholera agglutinins. With the exception of one, in which other causes might have operated in bringing about the high titre of 1 in 2,500 for the Inaba strain, the sera of all those that had no recent cholera inoculations were uniformly negative. The cases that were occurring were clinically cholera.

The effect of bacteriophage on the agglutinable vibrio.

The 12-1 phage type resistant cultures and the culture resistant to all 12 types were examined. It was found that there was a definite change in all to the rough state and H-agglutinability was considerably impaired or lost. Fermentation reactions were unimpaired when tested on solid media containing the sugars, but by the usual fermentation tests, results were not constant.

When the agglutinable vibrio was added to sterilised water containing phages and some organic matter, an agglutinable vibrio of fermentation type I could be recovered for 7 and 17 days in two experiments, after which no growth resulted. The duration of life of the vibrio was cut short by the phage but it had not undergone any unrecognisable change.

Selective cultivation of the Ag vibrio from mixtures of Ag and Nag vibrios.

Selective cultivation of the agglutinable vibrio from mixtures was not improved by growth in an atmosphere of 10-20% Co_2, anaerobic cultivation, growth in a heat-killed peptone water culture or filtrate of a peptone water culture of NAG vibrio and by some other methods tried. Over 30 chemical substances tried in different dilutions also failed to have any selective action on the agglutinable vibrio.

Viability of the cholera vibrio in natural water sources.

Two experiments were performed, where a culture of a recent agglutinable vibrio was added to a natural collection of water after its salinity had been adjusted and attempts made to recover the vibrio by the usual alkaline peptone water enrichment and the modified Wilson-Blair method. In the first experiment, the water source was a shallow well. After adding salt, the salinity was 26 parts Cl per 100,000 the pH being 5.1. The agglutinable vibrio could not be recovered even in the 24 hour sample. There was no natural vibrio in the water and rapid loss of salinity by diffusion was noticed.

In the second experiment the culture was added to a large tank of water. The pH varied between 6.3 and 6.7 and the Tidy figure for organic matter was 0.12 parts per 100,000. The salinity was adjusted to 70 parts per 100,000. The agglutinable vibrio was recovered in the 24-hour sample but not thereafter.

As the work was done during the end of October and the middle of November, when low temperatures prevailed, it is possible this factor contributed to the shortness of the period of viability of the vibrio.

7. CHOLERA TOXIN AND IMMUNITY ENQUIRY UNDER DR. H. GHOSH.

Studies were made on toxins of *V.cholerœ* prepared according to several different methods and tests were carried out on different laboratory animals by means of intracutaneous and intravenous injections. Preparations made by freezing and thawing a suspension of *V.cholerœ* and subsequently filtering so as to obtain a possible endotoxin and by filtration of broth cultures of various periods of growth, were found to be highly toxic. Similar toxic preparations were obtained when the organism used was an inagglutinable vibrio. At the same time filtrates of broth cultures of *B. coli* were found to be highly toxic when injected intravenously in rabbits. The work was unfortunately held up on account of shortage of experimental animals. The chemical characters of cholera stools were also studied and indicated a heavy elimination of NaCl. High alkalinity was found in all cases.

RECOMMENDATIONS REGARDING PRESENT ENQUIRIES.

The Committee having considered the reports of the several associated enquiries note that the phase of mainly laboratory investigation during the last five years has established a basis which should enable a field enquiry on the endemiology and epidemiology of cholera to be undertaken on satisfactory lines. The widespread distribution of vibrios, other than *V. cholerœ*, and the common prevalence of strains agglutinable by means of the type of cholera diagnostic serum formerly in use but not conforming to the other characters of *V.cholerœ*, which have been shown in water and other sources in India, are factors which in the past would have seriously complicated investigations. The evidence now available as to the result of the work of the past 5 years makes it possible to eliminate from consideration in the aetiology of cholera vibrios of the innumerable O serological types other than those of O group No. I which are so frequently isolated from cases of clinical cholera, healthy individuals and water in India.

A basis has been established for the identification of the serological type of vibrios which can be considered to be cholerigenic, and the study of the distribution and prevalence of this organism in endemic and epidemic cholera areas in relation to possible factors concerned in its survival and growth can now be undertaken under conditions in which a definite advance may be hoped for.

It is recommended that the investigations in cholera should now be directed mainly towards field study of factors concerned in the endemiology and epidemiology of the disease.

The Board therefore makes the following recommendations :—

A. In order to implement the above recommendation it is recommended that the enquiries now existing at

1. Central Research Institute, Kasauli,
2. School of Tropical Medicine, Calcutta,
3. All-India Institute of Hygiene and Public Health,
4. Pasteur Institute, Shillong,

should in a large measure cease on the present basis, although certain ancillary lines of work may be provided for in relation to a field enquiry in Bengal

which should be established for the purpose of investigating the factors concerned in endemic prevalence and epidemic spread of cholera. For this purpose some portions of the staff and the resources now available with those enquiries should be utilised and combined under one control. The enquiry should investigate the conditions of prevalence of true. *V.choleræ* in the local population and external sources in relation to geographical, climatic, and other factors, including the chemical composition of water and the epidemiological circumstances. In addition attention should be directed to the question of evidence of immunity in the population. This work should be carried out in areas presenting contrasting conditions. The enquiry should be actually established in the field with its own laboratory. An estimate is submitted for the cost of this at Rs. 32,900, the grant of which is recommended.

B. It is recommended that the cholera enquiry under the Director, King Institute, should be continued. This enquiry should, however, become a field enquiry and undertake the investigation of the factors concerned in endemic prevalence of cholera in the Cauvery delta area on lines similar to those of the field enquiry in Bengal. A budget provision for this enquiry at Rs. 8,020 is recommended. It is considered advisable that the officer in charge of the field enquiry in Madras should have a preliminary period of association with the workers undertaking the Bengal enquiry in order to establish common methods of procedure and technique.

C. It is recommended that the statistical enquiry under the Director, All-India Institute of Hygiene and Public Health, Calcutta, should continue on a modified basis to cooperate with the Bengal field enquiry. A budget provision of Rs. 2,620 is recommended for the purpose.

D. A grant of Rs. 3,124-9-0 is recommended for the continuance of the cholera vibrios enquiry at the Central Research Institute, Kasauli, for auxiliary work in connection with the preparation of sera and testing of strains of unusual character which may be isolated by the field enquiries.

E. As it is desired to complete certain lines of laboratory investigations now in progress at the Calcutta School of Tropical Medicine under Major C. L. Pasricha, I.M.S. and the withdrawal of a grant at the present stage would result in the loss of the work that has already been done and is unfinished, it is recommended that the grant of Rs. 6,120 is given for the coming year. This work includes a study of improved methods of isolation of *V.choleræ* from cholera cases studies in toxic strains, studies of the biochemistry of blood and urine in cholera cases.

F. Enquiry at the Pasteur Institute, Shillong, is not recommended for continuance.

The total under the heads of these associated enquiries which is recommended should be given is Rs. 46,664-9-0 which includes Rs. 40,920 for field work and Rs. 5,744-9-0 for work ancillary to the field enquiries. In addition Rs. 6,120 for laboratory work to complete lines at present in progress is recommended.

G. Cholera toxin enquiry.—As this enquiry has only been in progress for six months and it is not yet possible to determine whether the work is likely to be successful it is recommended that the enquiry be continued for one more year on the present basis with a grant of Rs. 3,800.

It is not recommended that the proposal for the preparation of anti-cholera serum for clinical trial be included as part of the work to be carried out under the grant from the I.R.F.A.

RECOMMENDATIONS REGARDING NEW ENQUIRIES.

A. Proposal of Dr. D. N. Bannerjee for an enquiry into the cholera kidney.

The proposal put up is for the study of the renal and extra-renal factors concerned in alteration of kidney function in cholera. The lines of work proposed in the extra-renal factors consist mainly in the study of blood changes. As work on this line is already in progress at the enquiry under Major C. L. Pasricha, I.M.S. at the School of Tropical Medicine, Calcutta, it is not considered advisable to recommend a grant for this purpose. A grant for the pathological study particularly of the kidney is recommended with the omission of provision for a biochemist which was included in the grant originally asked for. The amount now recommended is Rs. 1,300 to cover the services of technical assistant, chemicals and contingencies.

B. Applications for a grant by the Director of Public Health, United Provinces.

No specific proposals have been submitted for this enquiry and no budget. Certain lines of work are suggested for investigation in connection with the elucidation of the mechanism of dissemination of cholera on the dispersal of large fairs and in the elucidation of the effects of anti-cholera measures in the field. The committee consider that the effective carrying out of such investigations should await the results of basic study of factors concerned, which will be undertaken by the field enquiries which are now recommended to be instituted. When further progress is made as the result of such work, an extension of investigations on the lines of those suggested by the Director of Public Health, U. P., should be undertaken.

CHOLERA VACCINE.

At the request of the Public Health Commissioner with the Government of India the committee considered the question of the sources of supply and the nature of anti-cholera vaccines used in the several Provinces of India.

The vaccines used in the different Provinces are obtained from :—

(1) Laboratory under the control of Provincial Governments or their Public Health Departments or

(2) Commercial firms.

The Committee note that the nature of the vaccines used is stated in all cases by the manufacturing laboratories concerned to be the same, *viz.*, suspensions from agar cultures preserved with 0.5% phenol ; strength—8,000 million per c.c., strains used in manufacture—smooth strains, in some cases recently isolated ; belonging to the serological O group No. I ; giving the fermentation reactions of Heiberg type I ; non-hemolytic. In some cases several strains are used. In most cases strains are of the Inaba sub-type but in Madras Presidency both Inaba and Ogawa sub-type strains are used, infections of Ogawa sub-type being frequent in that Presidency. The practice in manufacture stated is in accordance with the recommendations made on the subject by this committee in 1936.

Examinations, however, which have been carried out on cholera vaccines available in the market in India have indicated that in certain cases the strains used in manufacture have not always been of the type specified. Certain vaccines have produced no agglutination response in experimental animals or men, and no protection in animals, and the vaccines themselves have been inagglutinable by O serum of group I type, while

on the other hand effective agglutination response, and full protection of animals, under the methods of test employed, has been shown by vaccines which were agglutinable. It would appear that vaccines giving such results cannot be accepted as being made from true cholera strains and no protection can be expected from their use.

The Committee wish to draw the attention of those responsible for carrying out anti-cholera inoculation to the necessity for assuring themselves that the vaccines they employ are manufactured from suitable strains in accordance with the recommendation of the Committee in 1936. For the purpose of ensuring that the vaccine is made from suitable strains it is recommended that the strains employed in manufacture in India should be strains tested and issued by certain specified laboratories, which have continuously engaged in the study of the cholera vibrio. The Directors of the Central Research Institute, Kasauli, the Calcutta School of Tropical Medicine and the King Institute, Guindy, have expressed their willingness to issue suitable strains to all laboratories, including those of commercial firms, for the purpose.

REPORT OF THE CHOLERA COMMISSION OF THE OFFICE INTERNATIONAL D'HYGIENE PUBLIQUE SUBMITTED AT THE SESSION OF MAY 1938.

The Committee having considered the report of the Cholera Commission note that the Commission have accepted in full the findings based on the investigations of workers in India on the serology of the vibrios. The Commission have expressed the opinion that the isolation from stools of cholera cases of vibrios which agglutinate with serum containing H agglutinins does not justify diagnosing them as *V.choleræ*. The test of O-agglutination on the other hand defines the vibrios to which a definite cholerigenic role may be assigned.

The Committee would like to take special notice of the finding that in a recent outbreak of cholera in the Celebes Islands of vibrios of the El Tor type were isolated from cases. The question of such strains playing an aetiological role in cholera is one of the highest importance in relation to the application of quarantine measures, and the opinion is expressed that a very full investigation on this subject is advisable together with further investigations on the subject of vibrio haemolysins.

REPORT OF THE NUTRITION ADVISORY COMMITTEE.

The Committee met on the 12th December, 1938.

1. PROGRESS IN 1938.

The Nutrition Advisory Committee, at its 1937 meeting, reviewed in detail the scheme of research and its application initiated at the first meeting of the Committee in June, 1936. It expressed its approval of the development of this scheme and recommended that work should be continued along the same lines. A detailed programme of further work was drawn up. The Committee notes that steady progress had been made in 1938 but considers that the time is not yet ripe for a further general review of the situation. The present report, which deals with certain questions of immediate importance, should be ragarded as an interim one.

2. STATUS OF THE COMMITTEE.

The Committee is gratified to note that the Government of India have decided to recognise it as the "National Advisory Nutrition Committee for India".

3. THE REPORT OF THE INDIAN DELEGATION ON THE WORK OF THE FOURTEENTH GENERAL ASSEMBLY OF THE INDIAN INSTITUTE OF AGRICULTURE, ROME (MAY 1938).

The Committee notes with satisfaction the resolutions on human nutrition on pages 20 and 21 of this report. It observes that, in general, the policy outlined in these resolutions with regard to the study of under-nutrition among rural populations and the relation between nutrition, agriculture and animal husbandry, is that being followed in India.

4. COLLABORATION WITH THE LEAGUE OF NATIONS.

The Committee recommends that the Coonoor Laboratories should undertake the task of collaboration in the nutrition work of the League of Nations, along the lines indicated in the note by Dr. Aykroyd which is included as an appendix to this report.

5. WHEAT.

The attention of the Committee was drawn to the following extract from page 26 of the Proceedings of the Second Meeting of the Wheat Committee appointed by the Imperial Council of Agricultural Research, held in 1937.

> "Mr. Fyffe drew attention to page 13 of Health Bulletin No. 32, recently issued from the Nutrition Research Laboratories, Coonoor. In this "balanced" and "ill-balanced" diets were given in which wheat products had been totally ignored in the "balanced" diet recommendation."

The Committee observes that wheat is on the whole a more nutritious cereal than rice or millet, and it regards the extension of the cultivation and consumption of wheat in India as desirable. At the same time, in many of the rice producing areas of India the partial or complete substitution of wheat for rice in the diet of poorer classes is not very practicable, because in many such areas wheat is relatively costly, unfamiliar to the people and cannot be grown. The present tendency of the well-to-do in South India to consume more wheat should be encouraged.

A paragraph dealing with the nutritive value of wheat should be inserted in the second edition of Health Bulletin No. 23.

6. INVESTIGATION ON RICE.

The Committee noted the progress of this investigation which was recommended in its last report. Approximately 200 questionnaires covering the subjects referred to previously were sent out and a detailed investigation of the vitamin B1 and mineral content of varieties of rice milled under different conditions is in progress. A detailed report is being prepared. In order that the conclusions contained in that report should have a wide circulation, it is suggested that a health bulletin based on this information should be issued under the authority of the Committee.

7. RED PALM OIL.

The Committee took note of a letter from the Adviser on Agriculture, Federated Malaya States, dated October 20, 1938, which referred to the

production of high grade red palm oil in Malaya and the possibility of using red palm oil as a cheap source of vitamin A in India. It was recommended that arrangements should be made to determine the vitamin A content of such oil in the Coonoor laboratories. Red palm oil is a cheap form of vegetable fat and the possibility of producing palatable and commercially suitable mixtures of red palm oil with other fats, particularly banaspati, should be explored. It was suggested that the question of preparing such mixtures might be investigated in the Harcourt Butler Institute, Cawnpore, the vitamin testing of the mixtures being carried out at Coonoor.

A study of the fruit of the palm *Elaeis guineensis*, from which the oil is obtained, is recommended, in order to discover which part of the fruit is richest in vitamin A activity. This investigation should be carried out in Coonoor with samples of fruit grown in South India.

8. Food production data.

In previous reports of the Committee attention was given to the problem of obtaining accurate data, collected by provincial agricultural departments, about the production of various foodstuffs with the object of discovering how far existing production meets the requirements of the population. The Committee, however, now considers that owing to the inaccuracy of the basic data, information so obtained is of limited value in the study and attack of nutrition problems in India. Except in the case of the chief cereals, production statistics are unreliable; this applies especially to pulses whose yields show wide local differences. It was agreed that the present method of carrying out limited diet surveys in sample areas was more likely to provide a true picture of food consumption and dietary habits in India and would be of greater use as a guide to agricultural policy.

9. Training courses for health officers in nutrition.

The Committee notes that the training course held in Coonoor in February-March, 1938, was attended by 15 health officers from States and Provinces in India. It recommends that a further course should be given in 1939 to suitable candidates delegated for training. It desires to bring to the attention of provincial and state administrations the great desirability of employing in public health nutrition work the officers who have already received this training.

10. Skimmed milk.

The Committee notes with satisfaction that the Government of India has accepted the recommendation made at its 1937 meeting, in respect of the customs duty on imported dried skimmed milk powder.

Having had its attention drawn to the increased sale of skimmed milk purporting to be whole milk, the Committee recommends that Provincial Governments and their public health organisations should consider legislative and other steps to prevent the sale of skimmed milk under the guise of whole milk. At the same time, constructive action to improve the supply of whole milk to cities and towns is even more urgently required.

11. Surveys in the Kangra Valley.

The Committee records its interest in the surveys carried out in the Kangra Valley by officers of the public health and agricultural departments, Punjab, under the direction of Lt.-Col. Nicol, and by Dr. D. C. Wilson and field workers attached to the Nutrition Research Laboratories, which were undertaken with special reference to the problem of rickets

and osteomalacia, which are common diseases in this valley. It considers that these surveys, which included studies of soil, economic conditions, diet and state of nutrition and the incidence of various diseases, represent a valuable contribution to the problem. It hopes that as a result it may be possible to find means of preventing the occurrence of rachitic conditions in this part of India.

12. The Liaison Officer's plan of work.

The Committee notes that the liaison officer has been working along the lines laid down in the 1937 report. He has visited Assam, Bengal, Bihar, United Provinces and Tehri Garhwal. During these visits he has made contact with agricultural departments and institutions and discussed problems of mutual interest with the agricultural officers concerned.

He sent out a questionnaire on the rice problem to marketing officers, etc. and has prepared a summary of replies.

A report on the nutritive value of pulses has been prepared. It is suggested that this report should be submitted for publication in one of the agricultural journals. A certain amount of laboratory work has also been done on the nutritive value of Indian diets.

It is recommended that the liaison officer's programme of work during the coming year should generally follow the lines laid down in the previous report. He should use his own discretion as to details. An investigation of the variation in mineral content of foodstuffs grown in different areas under varying conditions of soil and climate should be undertaken. Steps should also be taken to bring to the notice of local agricultural, public health and other departments the results of diet surveys carried out in various parts of India, and suggestions given for improvements in food supply applicable to local conditions. It is hoped to review the functions of the liaison officer and consider his programme of work in greater detail at the next meeting.

13. Existing and proposed enquiries.

(*a*) The Committee recommends the continuation of the work of the Nutrition Research Laboratories, Coonoor, and the Additional Nutrition Enquiry.

(*b*) The Committee recommends the continuation of the enquiry on cheap balanced diets under the Bombay Presidency Baby and Health Week Association provided that the work undertaken is planned in consultation with the Statistical Officer of the I.R.F.A.

(*c*) As Dr. Orr is not returning to India, the peptic ulcer enquiry is discontinued.

(*d*) Investigation on basal metabolism in children and adults in the Bombay Presidency by Prof. S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay. It is recommended that this enquiry be continued.

(*e*) Ascorbic acid enquiry by Dr. B. C. Guha at the University College of Science and Technology, Calcutta. It is recommended that this enquiry be continued.

(*f*) Scheme for food analysis by Dr. K. P. Basu, Dacca University, Dacca. It is recommended that this enquiry be continued and that an additional chemist be provided.

(*g*) Researches on the working efficiency of Indian labourers under Prof. W. Burridge, D.M.M.A.(Oxon), Professor of Physiology, King George Medical College, Lucknow. It is recommended that this enquiry be continued.

(*h*) Enquiry into malnutrition by Prof. N. M. Basu, Professor of Physiology, Presidency College, Calcutta. It is recommended that this enquiry be continued.

(*i*) Investigation by Dr. D. C. Wilson into level of blood phosphorus and serum calcium among cases of late rickets and osteomalacia in India in relation to chemical symptoms, treatment and cure. It is recommended that this enquiry be continued.

(*j*) Enquiry into the detection of adulteration of foodstuffs by the Public Analyst, Assam, at the Public Health Laboratory, Shillong. It is recommended that this enquiry be continued.

(*k*) Nutritional investigation on Bengal fish by Dr. B. C. Guha, at the Institute of Science and Technology, Calcutta. It is recommended that this enquiry be continued.

(*l*) Investigation on calcium metabolism in health and disease by Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay. It is recommended that this enquiry be continued.

(*m*) Tissue culture enquiry by Dr. G. Sankaran, at the All-India Institute of Hygiene and Public Health, Calcutta. The Committee recommends the parts of this enquiry which have a bearing on nutritional problems, but does not consider that it is capable of assessing the value of the whole proposal.

(*n*) Enquiry on malted cereals as infant foods by Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta. The Committee recommends that this enquiry be undertaken.

(*o*) Assessment of malnutrition of Bengali boys with regard to vitamin A deficiency by Prof. N. M. Basu, Presidency College, Calcutta. The Committee recommends that this enquiry be undertaken provided the necessary apparatus can be borrowed from the All-India Institute of Hygiene and Public Health, Calcutta. It does not recommend the purchase of apparatus.

(*p*) Investigation into the problem of dental caries in India by Dr. Rahman Siddiqui, School Health officer in the United Provinces. The Committee does not recommend Dr. Siddiqui's proposal that he should be employed by the Association as a full time investigator. It does, however, recommend that provision should be made for some technical and clerical assistance to enable Dr. Siddiqui to make some observations on this problem in the course of his work as a school medical officer.

(*q*) Nutritional and biochemical investigations in different varieties of rice by Dr. K. P. Basu, Dacca. The Committee does not recommend this enquiry.

(*r*) Researches by Dr. N. K. Basu of Dr. Bose's Research Laboratory, Calcutta, on certain problems of nutrition in India. The Committee does not recommend this enquiry.

Appendix

The collaboration of the Coonoor Laboratories in the Nutrition Work of the League of Nations.

The work of the League of Nations in the field of nutrition has been among the most successful of its non-political activities. It is to a great extent the result of its work that national nutrition committees have been established in many countries and that nutrition has become an important branch of public health activity. At the beginning the problems which were given most consideration were those of Europe and of countries with similar dietary customs. Recently, however, a number of League of Nations Commissions and Committees have recommended the extension of work to the Far East and the tropics generally. This proposal received the approval of the Council.

Nutrition was given an important position on the agenda of the Inter-Governmental Conference of Far Eastern Countries in Rural Hygiene held in Java in 1937 under the auspices of the Health Organisation of the League of Nations. The Conference, in its report on nutrition, recommended that "the Health Organisation should undertake to establish contact between the Technical Commission on Nutrition and nutrition workers in the East". With this end in view, the Health Organisation, meeting in February 1938, recommended that two workers in the East, Dr. A. G. Van Veen (Netherlands Indies) and Dr. W. R. Aykroyd should be appointed members of the Technical Commission. Both workers accepted the invitation. Dr. Van Veen attended a meeting of the Commission in November, 1937 and Dr. W. R. Aykroyd attended a meeting of a "Special Committee" under the chairmanship of Sir Edward Mellanby, at Geneva in August, 1938. The Health Committee also suggested that, with the approval of the competent authorities, the Coonoor Laboratories might collaborate in the development of nutrition work in the Far East.

The Coonoor Laboratories are occupied in a wide scheme of nutrition research and public health work extending throughout India. It is essential that this scheme should not suffer as a result of onerous work for the League of Nations. At the same time, very considerable benefit is derived from collaboration with a body such as the Technical Commission of Nutrition, which includes most of the leading nutrition workers of the world. Further, it is something of an honour to the Laboratories and the I. R. F. A. that the Coonoor unit should be specially asked for its collaboration in this matter.

At the meeting of the Special Committee in August, 1938, it was pointed out by certain members that the contact which had already been established between the Technical Commission and workers in the East ——*i.e.*, the attendance of Committees by Drs. Van Veen and Aykroyd—— had proved valuable in re-orienting certain ideas held by the Commission. It must be realised that many of the workers who are members of the Technical Commission are not very familiar with nutrition problems outside Europe and Northern America.

The Technical Commission is at present mainly concerned with certain aspects of nutrition. It is studying the dietary habits of populations in various countries and the best methods of carrying out such studies. It is interested in the problem of defining optimum dietary requirements for the various age groups and that of assessing "state of nutrition".

Methods to be followed for the practical application of the results of nutrition research——*e.g.* the development of public health nutrition work, the orientation of agriculture in accordance with knowledge of dietary requirements, etc.,——are of great interest to the Technical Commission and other League of Nations Committees concerned with the same subject.

The Technical Commission, in the report on the work of its third session in November, 1937*, suggested certain problems for study in the East. These are :

1. The composition of foodstuffs peculiar to the Far East.
2. Dietary surveys.
3. Dietary standards.
4. Assessment of the state of nutrition.

It has further suggested certain "problems previously noted for study by the Commission which might also be investigated in the Far East". These are :

Assessment of the nutritional state of children.
Minimum vitamin and mineral requirements.
Minimum fat requirements.
The nutritive and "supplementary" values of the different protein-containing foods to determine to what extent and in what forms protein is necessary for growth and health.
The relative nutritive value of different cereals according to the degree of milling.
Influence of climate on food requirements.
The extent to which diet in common use fall below the standards recommended in the Commission's report on the "Physiological bases of nutrition".

Now it is perfectly true that any work carried out in Asia, which deals with these and other related subjects, is eventually published in various journals and reports and thus becomes available to anyone in Europe who is interested. In practice, however, such work may escape the notice of the Technical Commission. It was therefore suggested to the Special Committee that the Coonoor Laboratories could supply the Technical Commission, with reports on work in Asia which is relevant to its own activities. In the case of work in India, this would usually mean forwarding reprints, or summaries of recent work, with perhaps an explanatory note drawing the attention of the Commission to various points.

At the same time, it is proposed to communicate with workers in other countries acquainting them with the activities of the Technical Commission and asking them to send relevant information and reports to Coonoor for forwarding to Geneva. At present there are only one or two laboratories and workers in countries outside India in a position to make contributions.

The Technical Commission at its future meetings may have to deal with other questions which would be clarified by knowledge of the position in the East. It is hoped that it may be possible to refer such questions to Coonoor for guidance. The procedure in this case would be to communicate with any other worker in the East likely to be able to supply useful information and forward his comments to Geneva together with remarks based on knowledge of Indian problems.

* Quarterly Bulletin of the Health Organization, 1938, Vol. VII, Extract No. 8.

The Special Committee expressed the view that the degree of co-operation outlined in the preceding paragraphs would be very valuable and hoped that arrangements could be made accordingly. No further suggestions for collaboration were put forward by the Committee.

In conclusion, it may be stated that on no account will activities in India suffer in consequence and that the scheme will be of benefit to nutrition workers in India. In practice the amount of work involved will be largely within the control of the Director of Nutrition Research.

REPORT OF THE LEPROSY ADVISORY COMMITTEE.

The meeting was held at 2-30 p.m. on the 14th December, 1938.

I. Lepers travelling on trains.

The part of the report of the meeting of last year, bearing on this question was read. Dr. Cairns explained how in view of the opinion there expressed, the Medical Section of the Indian Railway Conference had modified their resolution on this subject for presentation to the railway authorities. This resolution advocated the removal of leprosy from the list of infectious diseases which debarred the person affected from railway travel. Dr. Cairns stated that while sympathising with the motive behind the resolution, the railway authorities did not adopt it.

II. Enquiry under Dr. R. G. Cochrane.

This enquiry has investigated the value of improving by the addition of skimmed milk, the diet of children under treatment for leprosy. The findings made so far indicate that in cases of neural type no apparent benefit was seen, but in cases of lepromatous type, the addition to the diet of skimmed milk accelerated progress under treatment.

Dr. Cochrane presented proposals for the continuance of this work, and for the inclusion in the enquiry of clinical, therapeutic, and animal experimentation, with only a small increase of the budget.

The committee recommended the continuance of this enquiry along the lines proposed.

III. Enquiry under Dr. Lowe.

Dr. Lowe presented the report of this enquiry which includes clinical, histological, bacteriological, immunological, epidemiological, and biochemical studies and animal experimentation. He made proposals for the continuance of the work along similar lines.

The committee recommended the continuance of this enquiry.

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 2-30 p.m. on the 12th December, 1938, and considered the reports on the research work on malaria carried out during the past year and the proposals for work during the ensuing year.

(i) Malaria Institute of India.

The Director amplified the report previously circulated on the work of this organisation and laid special emphasis on certain features. The name of the organisation has been changed from 'Malaria Survey of India'

to 'Malaria Institute of India'. This was done on account of mis-conceptions which had frequently arisen among both laymen and medical men regarding its functions, which are considered to be more correctly indicated by the new title.

A number of tours have been undertaken by officers of the Institute in connection with the rural anti-malaria schemes which are being carried out in various provinces and to inspect the work of the field research units of the Institute.

During his tour in the Wynaad in May Lt.-Col. Covell was accompanied by Dr. Ramsay of the Ross Institute who kindly arranged demonstrations of the control work carried out in the tea estates in this area. Close co-operation is maintained between the staff of the Malaria Institute unit and the personnel of the Ross Institute working in this area, each benefiting by the results of the other's investigations.

Lieut.-Colonel Covell subsequently visited Paddukottai where the Rockefeller Foundation research unit under Dr. Paul Russell is carrying out important investigations into malaria problems connected with irrigation.

In the course of a subsequent tour in Southern India, Lieut.-Colonel Covell brought over the civil surgeon and anti-malaria officer of Coorg to see the control work in the Wynaad. The malaria problems in these two areas are very similar, and here again the co-operation of workers in each is of mutual benefit to both.

A conference of Chief Medical Officers of railways was held in November, and Lt.-Col. Covell took this opportunity to make contact with these officers in connection with railway malaria problems. It is hoped that with the transfer of the field station to Delhi a still further degree of co-operation with the railway medical departments will be secured.

Dr. Puri whilst on leave in Europe made a study tour in Italy, Albania and Holland which was financed by the Rockefeller Foundation, to which grateful acknowledgment was made. Dr. Puri also represented the Government of India at the seventh International Congress on Entomology at Berlin and at the Third International Congress on Malaria in Amsterdam.

A unit under the direction of Major Afridi carried out a malaria survey of Bahrein Islands (Persian Gulf) in May and June. Major Afridi also made a mosquito survey of Hog Island (Bombay) and visited Karachi to demarcate the area round the aerodrome for mosquito control. He has recently visited Kutch, where the work carried out with great attention to detail on his last year's recommendations has already yielded results of much value.

The annual malaria class for medical officers was held as usual, and a tribute was paid to the very useful work which is being done by many medical men who have received training in previous years throughout India.

New editions of three malaria bulletins have been published. New editions of two others are in the press. Three new bulletins are also in the press, one dealing with larvivorous fish, one with the prevention of malaria incidental to engineering construction, and the third with the cost of malaria to the Indian Empire. In addition to other advantages these bulletins serve as a text-book for the annual malaria class and it is possible to keep them continually up-to-date by the issue of new editions, which would not be possible if all the information were included in one large volume. There are now thirteen of these bulletins and taken together

they represent a text-book of more than 1,000 pages. A chart showing the action of quinine and synthetic drugs on malaria, based on the League of Nations Malaria Commission's Fourth General Report has also been published. There has been a very large demand for this. Cyclostyled notes on herbage packing and on the spraying of dwellings with insecticides have been widely circulated.

The scientific material supplied out to other workers included a number of strains of monkey malaria sent to the United States of America.

Among the insecticides tested experimentally there was a sample of dried pyrethrum flowers grown in Kulu which after soaking for 72 hours in kerosene oil gave very encouraging results. It is not possible at present to say what are the commercial possibilities of pyrethrum grown in this area, but the results indicate that it may be possible to produce a very cheap and efficient mosquitocide in India in the course of the next few years.

The anti-malaria operations in Delhi have again been supervised by officers of the Institute. Close contact has been maintained with the Central Public Works Department in carrying out certain engineering works remaining over from the 1937-38 programme. The reduction in malaria incidence has been marked, but control measures have been greatly aided by the deficiency of monsoon rainfall.

Old Flagstaff House, Delhi, has been allotted by the Government of India to accommodate the Field Station of the Institute. This will relieve the organisation of a burden which has greatly hampered its activities in recent years. It is expected that the transfer will be completed within the next few weeks.

As regards the field researches, *A. stephensi* has been incriminated as the principle and probably the sole malaria carrier in Bahrein Islands. The field research unit in the Wynaad has collected a mass of very valuable data. *A. fluviatilis* has been proved to be the chief malaria carrier, with an infection rate of over 20 per cent. during the transmission season. A field research unit has been working in the U. P. Terai since May. Preliminary investigations indicate that the problem there is of greater complexity than in the Wynaad, and the part played by the various potential malaria-carrying mosquitoes in this area has not yet been determined. In Delhi province the principle research activity has been in connection with the spraying of dwellings with insecticides. It has been shown that this is a method capable of application in villages and it represents our most hopeful method of dealing with epidemic malaria in this part of India. The study of the bionomics of *A. culicifacies* in the villages round Karnal has been continued.

As regards laboratory researches an important feature has been an intensive study of the behaviour of malaria parasites of monkeys, by the extra-abdominal spleen technique. Another line of research which has yielded results of great importance has been the carrying out of precipitin tests to determine the blood preferences of mosquitoes collected by the various field units.

Regarding the proposals for work during the ensuing year, the present lines of teaching and investigation will be continued and further developed. In the two foothill areas in which research units are working it is hoped to carry out various naturalistic methods of control, especially herbage packing and flushing of streams. For the latter purpose it is proposed to test an automatic sluice, a modification by Dr. Macdonald of

the Ross Institute of a type used in Malaya which has been very successful in Ceylon. Should this type of sluice prove as successful in this country as in Ceylon, it may revolutionize conditions of life in foothill areas in India.

It is proposed also to send a field unit to Orissa to investigate malaria conditions along the margin of Chilka Lake. There have been a number of serious epidemics of malaria in this area during recent years which constitute a matter of grave concern to the Orissa Government. The unit will consist of a sub-assistant surgeon, a laboratory attendant, four insect collectors, a sweeper and a peon. Co-operation has been promised by Mr. Parija, the distinguished botanist, in studying the surface *algae* of the Lake which are thought to have an important bearing on the malaria problem in this area. It is hoped later to include studies in the parts of the deltaic region which will have a general bearing throughout malaria-ridden areas of Bengal, Orissa and Madras.

The lines of work will be similar to those employed by the existing field units, namely the incrimination of the vector species of anopheline, the study of the bionomics of these species, epidemiological studies, the study of malaria among the local population (determination of spleen rates, parasites rates, species of parasites, intensity of infections etc.) and the formulation of a malaria control programme based on the results of the above investigations.

Major Mulligan drew special attention to the field researches which were a notable feature of the work of the Malaria Institute of India during the past year. He considered it very gratifying to note that the laboratory researches extending over a number of years were now being applied to the field. The lack of practical work has been frequently criticised in the past. The Directors of Public Health are now given expert guidance and advice in their schemes and with their collaboration rural anti-malaria control is now undertaken in India on a scale which is second to none in the world.

Colonel Jolly wished to associate himself with the desirability of this new departure in the programme work carried out by the Institute. He considered that the combination of laboratory researches with the practical demonstration of their application in the field is excellent.

The Committee approved the proposals put forward for favourable consideration by the Scientific Advisory Board.

(ii) MALARIA TRANSMISSION ENQUIRY.

In the absence of Dr. B. M. Das Gupta the report of this enquiry previously circulated was explained by Colonel Chopra.

The investigations carried out during this year concerned 3,997 laboratory-bred clean female *A. annularis*. These were subjected to ten different combinations of temperature and relative humidity in the air-conditioned cabinet and the percentage of infection amongst them was determined. The results of work on *A. stephensi* have now been analysed and kept for comparison with those obtained with *A. annularis*.

The effect of 'Cilional' on the infectivity of patients to mosquitoes was also studied and a report published in the *Indian Journal of Medical Research*. Another report of this subject was under publication in the next number of the *Journal of the Malaria Institute of India*.

The chemo-therapy of anti-malarial drugs in general has been studied under the direction of Colonel Chopra both in human and monkey malaria.

Special efforts have been directed towards determining the efficacy of Cilional and of the sulphanamide group of drugs.

In addition to the above malaria researches, investigations have been carried out on Weil's disease and two different strains of the organisms of this disease have been isolated from cases discovered in Calcutta.

In the ensuing year it was proposed to study the factors governing the infectivity of *A. culicifacies* and other important malaria-carrying species of Anopheles available in Calcutta. Work would also be undertaken in connection with (*a*) the effect of anti-malarial drugs upon the sporogony cycle of malaria parasites in mosquitoes, (*b*) investigation of the chemo-therapy of anti-malarial drugs, (*c*) certain aspects of the biology of the sporogony cycle of malaria parasites and (*d*) further studies on Weil's disease.

The committee approved the proposals in so far as they relate to malaria and recommended the continuance of the enquiry.

(iii) INVESTIGATION INTO THE MAXILLARY INDEX OF A. STEPHENSI IN CALCUTTA.

Owing to the departure of Mr. Senior White on leave ex-India this enquiry, with the permission of the Secretary of the Indian Research Fund Association was suspended on 31st May. The report therefore is confined to the results obtained during the five months from December 1937 to April 1938.

The captures of *A. stephensi* made from various situations showed that the highest prevalence of this species was in February and June. As meteorological conditions of these months are dissimilar, it is surmised that variations in numbers are not occasioned by this factor.

In an effort to ascertain the preferred resting places of this species in Calcutta certain investigations were made. Human bait traps did not yield catches of sufficient numbers nor were successful results obtained from spraying rat holes and ceilings. This problem therefore still remains to be solved.

Up to April 30 a total of 115 maxillae have been counted from those mosquitoes caught in houses or trapped on man, and 220 from those caught in cattle shed or trapped on cows.

Until the full desired number of 200 in each set is obtained, no reliable conclusions can be drawn from these results.

Efforts were made to differentiate the 'B' and 'M' forms of *A. stephensi* prevailing in Calcutta, and the results so far show that the predominating race is 'B'. The maxillary counts of these races are now being recorded separately. Further work is however necessary to determine to show whether the 'M' form prevails in sufficient numbers to vitiate the results of the counts reported previously.

The habits of the common mosquitoes of Calcutta as revealed by human and cattle trap catches were studied. The figures obtained indicate that all species of anophelines show a preference for cattle over man, more especially *A. stephensi*. Among the culicines, with the exception of *Culex fatigans*, which has an equal attraction for man and cattle, the other species display a similar preference.

The report was approved by the Committee, who recommend that the enquiry be resumed from February 1939 on the return of Mr. Senior White from leave.

(iv) BLACKWATER FEVER ENQUIRY.

The report of this enquiry, previously circulated, was further explained by Dr. Krishnan. He pointed out that during this year five more human cases of blackwater fever were treated with a combination of cortin, ascorbic acid and glucose. This form of treatment had been evolved out of the biochemical and pathological studies of blood and tissues of monkeys developing haemoglobinuria. The uniform success so far attained in treating blackwater fever by this method was considered to be encouraging especially as none of the cases were known to have relapsed hitherto. Reference was made to the difficulty of procuring a sufficient number of cases for such treatment, inspite of posting assistants in the affected areas.

In addition to these studies attempts were made to elucidate the role of vitamin C in malaria, especially with a view to preventing the development of blackwater fever. A small hyperendemic malarial village in Bengal was selected for the experiment, and a comparison of the healthy and the malarial groups of the population was made with regard to vitamin C saturation. After ascertaining the "normal rate", a test dose of ascorbic acid varying with the body weight was given, and the rate of excretion of the acid during the ensuing 24 hours ascertained. The results showed that the majority of the healthy children between the ages of 2 and 10 had a normal vitamin C saturation, but in adults there was a condition of hypovitaminosis C. During an attack of malaria there was increased excretion of ascorbic acid in the urine, both amongst the children and the adults, while during afebrile intervals the excretion was markedly less. In chronic malarial subjects there was a well marked sub-scurvy state which was rendered more intense by each attack of fever. This was especially noticeable in malarial subjects belonging to a low economic status. Adequate diet and the administration of vitamin C by the mouth improved the condition of hypo-vitaminosis C.

Rotter's intradermal test was also tried to confirm the above findings, but the results obtained were inconsistent and unreliable.

At present the ascorbic acid content of plasma in the different groups was under investigation.

The Committee approved the proposals and recommend the continuance of the enquiry.

(v) ENQUIRY ON THE PHARMACOLOGY OF ANTI-MALARIAL DRUGS.

The report of this enquiry previously circulated was further amplified by Dr. Dikshit. He pointed out that the action of plasmoquine and atebrin did not affect the course of pregnancy. Investigations during the present year have been made on the action of these drugs on the growth and behaviour of the fœtus. This suggested that the placenta probably acts as a filter. No interference was detected when the drugs were given in therapeutic doses. To elucidate this point records of the heartbeat and respirations of the fœtus were made by means of a special technique. There was no action on these organs in the foetus, even when large quantities of the drug were circulating in the maternal blood. This was confirmed by quantitative estimation of the drug in the fœtal and maternal blood simultaneously. Only very small quantities of the drug were passed on to the fœtus, and no injury to the organs resulted.

The placenta appeared to be a less effective barrier to atebrin than to plasmoquine.

Investigations were also made as to the fate of plasmoquine in the body. This aspect of the investigation is still incomplete and is being pursued. The results so far obtained show that plasmoquine is rapidly destroyed in the body and that the organs chiefly responsible are the liver and the brain. The process of destruction is probably of the nature of enzyme action, as the acetone extract of these organs destroys plasmoquine.

The fate of atebrin in the body has not been sufficiently investigated. Preliminary observations show that only a very small portion of the drug remains in the blood for seven hours and that it disappears completely in 24 hours. This concentration in the blood does not go beyond a certain limit without injurious action on the heart.

The greater portion of the drug passes almost immediately into the tissues of the body. Dr. Dikshit proposed to continue his enquiry on similar lines in the following year.

The Committee approved the report and recommend the continuance of the enquiry.

(vi) INVESTIGATIONS UNDER THE ASSAM MEDICAL RESEARCH SOCIETY.

Dr. Rice amplified his report on the activities of this Society. He pointed out that surveys of three new areas had been undertaken in addition to that of an extensive Bhil area commenced in 1937, which was completed during this year.

He emphasised the extension and scope of the malaria control projects carried out in Assam. These were supervised by members of the society staff, who had previously surveyed and made recommendations on the areas. Thus in addition to 6 experimental anti-larval control centres, 138 other centres receiving financial assistance from the Government of Assam were supervised. Similarly periodical visits were paid to seven tea estates where the Society's recommendations for the control of malaria have been implemented. Moreover in co-operation with the Public Health Department and the Provincial Advisory Malaria Committee, supervision of the anti-larval scheme in the provincial areas was continued.

Studies on the cold weather bionomics and pre-monsoon malaria conditions were continued at the experimental anti-larval control centres. In two centres the institution of pre-monsoon control brought about a significant decrease in the incidence of the disease. The previous observations made on pre-monsoon control of mosquitoes breeding were thus confirmed and the evidence is now very strong in favour of the utility of this method in Assam.

The Doom-Dooma cinchona-plasmoquine treatment scheme has been continued for the sixth year. The high parasite rates reported during the year 1936 were not observed this year and actual reduction in the rates occurred. Many tea estates have now adopted the method of treatment demonstrated by these experiments.

Two groups of villages were selected for treatment with reinforced cinchona febrifuge. These were financed jointly by the Government of Assam and the Local Board.

Apart from the researches outlined above, a great deal of attention was paid to preventing larval drift, and the methods for bringing about satisfactory results were improved and rendered more effective than heretofore.

The Committee expressed its appreciation of the work carried out under the direction of Dr. Rice.

The resolutions on malaria by the 15th Conference of Medical Research Workers were considered by the Committee, which noted with satisfaction that appropriate action had been taken on them.

The following resolution was accepted by the Committee and recommended for consideration by the Conference :—

Considering the importance of malaria as a cause of mortality and disability in India the Committee recommends that provision be made to admit a larger number of medical men for training in the annual malaria course held by the Malaria Institute of India.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met on 13th December 1938.

I. Plague researches at the Haffkine Institute

The Committee considered the report on the research work on plague carried out at the Haffkine Institute, Bombay, and heard a statement on the subject.

(*a*) *Anti-plague vaccine and the study of antigenic structure of B. pestis.*

Owing to certain unavoidable delays in commencing the chemical work on the study of antigenic structure of *B.pestis*, it has not been possible yet to present positive findings on the subject. The preparations of extracts of the protein, nucleo-protein and polysacchands fractions of the organism is in progress and the results of tests of their properties will be awaited. The Committee considers that, parallel to this line of investigation, a study of antigenic properties by serological methods should be carried out and that this should include a comparison of strains which are (*a*) virulent and protective when used for immunization, (*b*) avirulent and protective, and (*c*) avirulent but not producing any protection. The Committee notes that it has been confirmed that agar vaccines grown at 37° C. afford higher protection than those grown at 27° C. and that it has been found that vaccines of uniformly good uniform protective power can be obtained by the use of suspensions from agar. The possibility of adopting an agar-grown vaccine for prophylactic use should be kept in mind and considered in the light of further experimental work.

(*b*) *Anti-plague serum.*

The Committee notes the results of the recent trials of the anti-plague serum on 69 cases of plague with 55 controls. An examination of the figures has been made by the medical statistician who states that "the conclusion that I am inclined to draw from these analyses is that anti-plague serum has been shown to reduce mortality from the disease". The total number of cases so far treated with serum is, however, not sufficient to afford a final conclusion as to the value of the serum treatment of plague. It is accordingly considered advisable that the trials should be continued on an experimental basis.

II. The Cumbum Valley enquiry.

The Committee discussed the Report of the Plague Recrudescence Enquiry in the Cumbum Valley. This is the only plague enquiry of this

nature at present being carried out under the I. R. F. A. and the opinion is expressed that much information is being obtained on practical methods of plague control. The further observations recorded during the year on the value of cyanide disinfestation and methods of its application will afford a useful guide to those undertaking control in rural areas by such means.

III. PROPOSALS FOR CONTINUANCE OF ENQUIRIES.

(*a*) It is recommended that the enquiry at the Haffkine Institute be continued on the lines of the proposals put up. The investigation should include (*a*) further studies on the antigenic structure of *B.pestis* in relation to the selection of strains for vaccine and the methods most suitable for manufacture, and (*b*) further trials of anti-plague serum on an experimental basis. For this purpose a grant of Rs. 32,109/- is recommended.

(*b*) It is also recommended that a grant of Rs. 4,500/- be given for the continuance of the Plague Recrudescence Enquiry in the Cumbum Valley. The Committee notes that the Travancore Government have agreed to give a contribution towards the cost of this enquiry and hopes that the Madras Government will see its way to continue their contribution. The Committee wishes to draw the attention of the workers in this enquiry to the special methods of study applicable to the bionomics of fleas, namely the method of breeding under controlled conditions of temperature and humidity which have been developed by Dr. Buxton at the London School of Tropical Medicine and Hygiene and recommends the adoption of such methods in further studies of the bionomics of fleas in relation to plague transmission.

(*c*) The Committee noted the communication of the Director of Public Health, Central Provinces, to the Director of Haffkine Institute on the subject of certain problems of plague, particularly the incidence of cases of mild type with very low mortality in that province. The problem is considered one of considerable interest and is recommended for study. The Director, Haffkine Institute, has undertaken to carry out an investigation of a preliminary nature with material supplied from the Central Provinces and to report on the findings. No grant is requested for the purpose.

REPORT OF THE MATERNAL MORTALITY ADVISORY COMMITTEE

1. The Committee met at 5-30 p.m. on the 12th December, 1938, and considered the proceedings of the sub-committee appointed last year to examine and, if necessary, suggest amendments to Dr. Neal Edwards' enquiry into the causes of maternal mortality in Calcutta. It was noted that the sub-committee considered that the value of the report to administrative officers will be enhanced by the addition of certain sections. The Committee agreed with this view and noted that Dr. Neal Edwards is at present preparing the additional sections. The Advisory Committee also agreed with the recommendation that the report in its final form should be referred to the Public Health Commissioner with a view to its publications as a health bulletin.

2. The Committee considered the note by Dr. Jhirad on her completed enquiry into the causes of maternal mortality in Bombay and decided that the full report, when prepared, should be submitted to the sub-committee which considered Dr. Neal Edwards' report. It was also decided

that while Dr. Jean Orkney, a member of that sub-committee, is on leave, her place should be taken by Dr. Neal Edwards.

3. The Committee considered the memorandum which had been prepared by the same sub-committee on the "Conduct of a maternal mortality enquiry". It was considered that the information provided in the memorandum will be of the greatest value to administrative officers who are desirous of undertaking such enquiries in their particular areas.

It is recommended that the memorandum should be referred to the Public Health Commissioner with a view to its publication and distribution to those authorities who are likely to be interested.

4. It was noted that the maternal mortality enquiry in a rural area in Bihar was not proceeded with this year owing to lack of an experienced obstetrician to check up the findings, to amplify the notes investigated, and to analyse the schedules.

5. The Committee were given an account by Dr. Napier of the work done in his enquiry at the School of Tropical Medicine into anaemia of women. The Committee are of opinion that the information collected in this enquiry is of the greatest value and recommend its continuation in the coming year.

6. The Committee considered the report by Dr. Napier on the work of the anaemia enquiry at the All-India Institute of Hygiene and Public Health. It was concluded that the enquiry has done useful work and much valuable data has been collected. The continuation of the enquiry on the present lines in the coming year is not recommended, as the next step should be the analysis of the findings. It is, therefore, recommended that the Scientific Advisory Board should set aside a sum to provide such clerical assistance as Dr. Napier may require next year in order to carry out this analysis.

7. The Committee noted that Dr. Benjamin's work at the Lady Hardinge Hospital, New Delhi, on the blood of normal women in Delhi Province has been completed and valuable information has been obtained which confirms findings in other parts of the country. It was noted that a full report will be submitted shortly.

8. The Committee considered Dr. Purandare's report on his enquiry into toxaemia of pregnancy in Bombay. Dr. Purandare gave an account of the findings in this enquiry during the current year. In the ensuing discussion, there was a divergence of opinion as to the advisability of recommending a grant for the continuation of this work. While some members supported it, others put forward the view that the work was rather of a clinical nature and such as might be carried on with the facilities already available in the hospitals concerned.

9. The Committee considered the note presented by Dr. Vishwa Nath on his investigation into the biochemical and dietetic factors concerned in the toxaemia of pregnancy. Dr. Vishwa Nath described to the Committee some additional findings of his enquiry. The Committee consider that a number of interesting findings have already been recorded and recommend the continuation of this enquiry next year.

10. The Committee considered the note presented by Lt.-Col. Hayes on the investigation at Lahore into the bacteriology of the genital tract. Lt.-Col. Hayes described the nature of the work and Colonel Taylor gave an account of the part played in the enquiry by the Central Research

Institute. There was no proposal before the meeting for the continuation of this enquiry next year. The Committee considered that in view of the vital importance of this subject, further investigation of the problem is most desirable. The Committee also considered that if research into this question is to be pursued, it would be a suitable subject for a co-operative enquiry between a Provincial Government and the Indian Research Fund Association.

11. The Committee heard statements by Dr. Neal Edwards and Major Crichton regarding the steps which have been taken to carry out in the urban areas of Delhi Province and in the Najafgarh Health Unit a maternal mortality enquiry on the lines followed in Calcutta. The Committee approved the plan of work and recommend the continuation of the enquiry in the coming year.

12. The Committee considered a proposal for a new enquiry by Dr. G. N. Vyas for an investigation of the clinical and haematological picture of pernicious anaemia in Indian patients including infants. The Committee felt that the study of anaemia in children is one which requires to be taken up, but could not see their way to recommending Dr. Vyas' proposal in its present form.

13. The Committee considered a proposal by Mr. J. C. Bardhan for the preparation of tetra-cyclic ketones related to oestrone starting from cheap coal-tar products, and the comparison of the therapeutic properties of the synthetic products with oestrone prepared by standard methods. The Committee considered that the subject is not directly related to maternal mortality and is of too technical a nature for them to be able to advise upon it.

REPORT OF THE TUBERCULOSIS ADVISORY COMMITTEE.

The Committee met at 2.30 p.m. on the 13th December, 1938 and reviewed the current year's work and the proposals for 1939-40.

(a) Enquiries under Dr. A. C. Ukil.

Dr. Ukil briefly explained the lines of investigations which are now being carried out under his enquiry.

(i) *Tuberculosis in relation to industry.*

After having made an intensive study of 263 workers selected from different departments of the Jute mills, as samples, according to standardised schedules, giving the previous habitation, duration of industrial life, physical and radiological examinations and blood tests, a serial examination of 1,150 labourers was done, including tuberculin testing and other examinations; home survey of 450 dwellings in barracks and contact survey of 12 houses containing active cases of pulmonary tuberculosis, many of which were clinically 'silent' cases, were also carried out. In order to ascertain the dust hazards in the industry, the size and concentration of suspended dust particles in different sections of the industry were obtained with the help of a Dust Counter. Dr. Ukil explained the methods of assessing chest skiagraphs which he was following in this enquiry. He proposed to include the total mill population in this investigation and to compare the industrial with some non-industrial groups. He also proposed to conduct a tuberculin survey of all the relatives

of the workers, particularly children to examine the contacts, if available, of all "definite" and probable cases, to examine a certain number of new entrants to the industry and to follow them up in their industrial life and to enquire into the conditions prevailing in some other industries with special tuberculosis and dust hazards.

(*ii*) *Tuberculosis in "home contacts" particularly among children.*

Dr. Ukil pointed out that up-to-date 827 children below 15 years had been examined clinically, tested with tuberculin and examined radiographically, home visits and survey being carried out at the same time. He pointed out that from the facts available he found tuberculosis mortality to be 50% higher among child contacts and 3½ times higher among adult contacts in the homes investigated than the rate of general mortality. He indicated that his studies in this line had brought out tuberculosis as an important cause of morbidity and mortality among infants and children, which has not hitherto been noticed in public health returns. He hoped to raise the number of cases to 1,000 and to make a study of some non-tubercular homes as controls during the next year.

(*iii*) *Typing of tubercle bacilli from pathological materials* (*non-pulmonary*).

Dr. Ukil pointed out that this work was now confined to the study of strains, which he had already collected, and to strains from rare non-pulmonary sources such as the skin. Out of 218 strains isolated, 193 strains have been typed out, proving all of them to belong to the "human" type. 25 strains are still awaiting typing.

(*iv*) *Tuberculin survey of the population of certain institutions, which were investigated by Dr. Ukil ten years ago.*

Dr. Ukil proposed to re-examine, by tuberculin testing, the people of the same institutions which he investigated ten years ago with a view to find out whether the incidence of tuberculous infection has materially increased in those areas in the course of the last ten years. He further explained that he needed only travelling allowances for his staff to the extent of Rs. 500/- and that no extra staff was needed for this investigation.

An interesting discussion took place in which several useful suggestions were made, particularly with regard to the survey of the families of labourers, periodical examination of new entrants and the necessity of taking note of workers who dropped out of the industry owing to tuberculous disease. Dr. Frimodt Moller emphasised the value of finding out and interpreting the susceptibility of Indians to tuberculosis by graded tuberculin testing and of using the Von. pirquit test in areas where the intradermic test could not be easily applied.

The continuance of Dr. Ukil's enquiry was recommended.

(b) ENQUIRY INTO THE TUBERCULOUS INFECTION AMONG MEDICAL STUDENTS AND NURSES OF THE K. E. MEDICAL COLLEGE AND THE ASSOCIATED HOSPITALS AT LAHORE BY MAJOR G. F. TAYLOR, I.M.S.

Major Taylor placed before the Committee his findings on tuberculin testing and on X-Ray and physical examination of students and nurses of the Mayo Hospital, Lahore, and of some schools and orphanages in the town. He also mentioned his findings on the examination of family contacts of active lung tuberculosis cases. His investigations showed up-to-date that 4.5% of the 400 medical students and 5.3% of 38 nurses had radiological evidence of healing or healed lung tuberculosis. He drew the

attention of the committee to a striking fact regarding the results of tuberculin test in the first year and second year students of the Lahore Medical College. The first year students gave double the number of positive reactions as compared to those in the second year. He had, however, failed to discover any source of infection in the Medical College to account for this apparent discrepancy, which might be due to the small number of students examined.

Major Taylor proposed to re-test all negative tuberculin reactors of the previous year, to repeat the X-Ray examination of tuberculin positive cases, to examine the new first year students and the students of a non-medical college and to follow up the medical history of these students as well as nurses, during the next few years.

In an interesting discussion which followed, it was suggested that those students might be traced back to their homes and institutions where they had studied with a view to find out possible sources of contagion contributing to abnormally high number of positives in any one class.

The committee recommended the continuation of this enquiry.

(c) TUBERCULIN SURVEY OF SCHOOL CHILDREN AND URBAN POPULATION AT AMRITSAR BY MAJOR S. M. K. MALLICK, I.M.S.

Major Mallick explained that he had to do a lot of propaganda work in order to induce school children and the people of a poor locality at Amritsar to submit to tuberculin testing, and hence the number of people tested has not been as large as he anticipated. He hoped to proceed with the work more quickly as he had now better arrangements.

After some discussion, the continuance of this enquiry was generally approved.

CONSIDERATION OF THE QUESTION OF ADDITION OF SOME SCHEDULES AND DEFINITIONS AS AN ADDENDUM TO THE REPORT OF THE TUBERCULOSIS SURVEY COMMITTEE, I.R.F.A. (1937).

As the matter required careful and detailed consideration, it was referred to a committee consisting of the following members :— Colonel G. G. Jolly, I.M.S. (*Chairman*). Dr. C. Frimodt-Moller, Dr. R. B. Lal, Dr. B. K. Sikand and Dr. A. C. Ukil (*Secretary*).

A GENERAL REVIEW OF TUBERCULOSIS PROGRESS IN INDIA DURING 1938.

The Chairman briefly referred to the notes circulated by Dr. Sikand, Organising Secretary, King George Thanks-giving (Anti-Tuberculosis) Fund as regards the work of the Fund and the progress achieved by the different provinces in anti-tuberculosis work in 1938.

The Chairman next referred to the function and scope of work of the proposed Tuberculosis Association of India and invited suggestions regarding some of its methods, particularly with reference to the notes prepared by Col. H. Stott, I.M.S., Inspector-General of Civil Hospitals, Bihar.

An interesting discussion took place in which Dr. Frimodt-Moller made some very useful suggestions for improving the methods of anti-tuberculosis work in the provinces, and he thought that such a scheme should include survey, research and training of doctors at selected provincial centres.

Dr. Frimodt-Moller was next invited to give his opinion on the function and scope of work of the proposed Tuberculosis Association of India,

particularly regarding propaganda, training, supply of information, co-ordination, research and acting as an advisory body for the provinces and states. He made some valuable suggestions on these points.

Administrative heads of the medical and public health departments from several provinces expressed their views with regard to the anti-tuberculosis measures, which could be put into operation in their own areas. It was realised that the problem while agreeing in general principles varied in detail in the different localities. It was felt that some suggestions in general outline regarding the methods to be followed in each area might be of help to the Provincial-State anti-tuberculosis organisations.

The Chairman referred to the tremendous interest in the anti-tuberculosis measures, which Her Excellency's Appeal for the King Emperor's Anti-Tuberculosis Fund, had raised throughout India and to the need for continued interest in the problem. He stressed on the necessity of close co-operation and co-ordination between the Provincial-State medical and public health departments as well as the general medical practitioners. The following resolutions moved from the chair was unanimously passed :

This Committee recommends the following resolutions for adoption at the 16th Medical Research Workers' Conference :—

> "This Conference approves of a note entitled 'The Tuberculosis Problem in India' which has been issued under the auspices of the King Emperor's Anti-Tuberculosis Fund and believes that it will provide useful guidance to Provincial-State anti-tuberculosis organisations."

RESOLUTIONS.

1. Considering the importance of malaria as a cause of mortality and disability in India the Conference recommends that provision be made to admit a larger number of medical men for training in the Annual Malaria Course held by the Malaria Institute of India.

2. This Conference approves of a note entitled "The Tuberculosis Problem in India" which has been issued under the auspices of the King Emperor's Anti-Tuberculosis Fund and believes that it will provide useful guidance to Provincial-State anti-tuberculosis organisations.

3. Owing to the increased incidence of kala-azar in Bihar, Bengal and Sikkim and to the persistence of this disease in Assam, this Conference recommends that an extended kala-azar enquiry under the Indian Research Fund Association be instituted to carry out investigations on the following lines :—

(*a*) A field survey of an intensive nature in a restricted endemic area,

(*b*) improved methods of prevention, and

(*c*) transmission of the disease.

Further, in view of the high mortality amongst untreated cases of kala-azar, and of the necessity for obtaining cheaper treatment and more effective means of prevention, this Conference expresses the hope that Provincial and other Governments will find it possible to contribute, as they did previously, towards the extended enquiry which is now recommended.

INDIAN JOURNAL OF MEDICAL RESEARCH & INDIAN MEDICAL RESEARCH MEMORIES.

During the year which has elapsed since the last Conference Volume XXV of the *Journal* has been completed by the publication of the January and April 1938 numbers, and the July and October numbers of Volume XXVI have appeared.

A. (1) *Volume XXV* (1937-38).—*The Journal.*—Four quarterly numbers of this Volume were issued, the average size being 248 pages of literary and tabular matter and 6 plates. This represents a decrease of 54 pages on the amount of matter published quarterly and a decrease in the number of plates by 4 in comparison with the previous Volume. No delays occurred in publication.

(2) *The Memoirs.*—One *Memoir* was published with this Volume, viz., No. 29—'The histopathology of malaria with special reference to the function and origin of the macrophages in defence' by W. H. Taliaferro and H. W. Mulligan (138 pages, 15 plates) (May 1937).

(3) The net cost of Volume XXV was Rs. 12,017. This is inclusive of cost of one *Memoir* (No. 29) published with the Volume (Rs. 2,369). This represents a decrease in cost as compared with previous Volume in spite of the fact that a *Memoir* was published.

B. (1) *Volume XXVI* (1938-39).—*The Journal.*—The July and October numbers have been issued containing respectively 327 pages and 9 plates and 267 pages and 1 plate (inclusive of 1 coloured half-tone and 3 coloured line-block figures in the July issue).

(2) *The Memoirs.*—One Memoir has been issued with this Volume viz., No. 30—'Studies in the epidemiology of filariasis in Travancore by M. O. T. Iyengar (179 pages, 12 plates) (July 1938).

C. *Distribution.*—

Indian subscribers	..	107
Foreign subscribers	..	157
Exchange list	..	170
Free list	..	117
Voucher copies to advertisers, file, regn :, police, make up, specimen copies to intending advertisers, propaganda, etc	..	40 (approx :)
Total	..	591
Balance left with publishers for sale	..	309
Total copies printed of the *Journal*	..	900

There has thus been an increase of 11 in the distribution but the paid circulation is still very small, the increase being only 5 over last year. The exchange list has been increased by 12 by addition of 6 new addresses and by transferring from the free list 6 addresses from which exchange publications are received regularly. A large number of publications are obtained in this way for the I.R.F.A. Library.

D. *Distribution lists.*—As there have been many changes and additions to the distribution lists of the *Journal* since they were last revised

in 1933, a new corrected alphabetical list showing exchanges has been printed and copies are now available from the office of the Secretary for distribution to workers under the I.R.F.A.

E. *Number of copies printed.*—This remains at 900 and it has been ascertained that there would be comparatively little saving by a reduction in the number. Spare copies of numbers of Vols. I to VIII are available for issue from the office of the Editor, *Indian Journal of Medical Research*, Kasauli.

F. *The cost of printing the Journal.*—The following statement shows the cost of production during the last five years :—

Cost of printing the *Journal only.*

Number.	XX-XXI 1933-34	XXI-XXII 1934-35	XXII-XXIII 1935-36	XXIII-XXIV 1936-37	XXIV-XXV 1937-38
April	4,632	4,063	4,330	3,369	4,468
July	3,580	3,436	5,058	4,459	4,907
October	3,320	3,277	3,893	3,931	4,622
January	3,660	2,649	4,160	5,141	4,572
Totals	15,192	13,425	17,441	16,900	18,569
Average pages	246	222	276	284	278

Recoveries.

April	2,311	1,840	1,593	1,881	1,534
July	2,078	1,105	1,593	1,318	1,534
October	507	1,017	1,659	1,318	1,185
January	1,782	1,656	371	1,466	1,185
Totals	6,678	5,618	5,216	5,983	5,438

G. *Net cost of the Journal and Memoirs.*—The following statement shows the net cost of the production of the *Journal* and *Memoirs* during the last five years :—

Journal.

April	2,321	2,222	2,737	1,488	2,934
July	1,502	2,332	3,465	3,141	3,373
October	2,813	2,260	2,234	2,613	3,437
January	1,878	993	3,789	3,675	3,387
Totals	8,514	7,807	12,225	10,917	13,131

Memoirs.

	No. 28 1,391	.. ..	.. ..	.. ..	No. 29 2,369
Totals	1,391	..	..	..	2,369
Grand totals	9,905	7,807	12,225	10,917	15,500

For convenience of accounting all receipts are credited to the *Journal* account and the figures given for the *Memoirs* are the actual cost of production.

The net cost during 1937-38 after deducting receipts was Rs. 15,500 out of which Rs. 2,275 was already with the publishers as advance from saving of 1935-36 for Memoir No. 29. All publishers' bills for 1937-38 were submitted in time to make payments within the financial year.

H. *Financial position for the year* 1938-39.—The budget allotment for the year was Rs. 13,000 for the cost of four quarterly numbers of the *Journal* plus Rs. 2,500 for any *Memoir* which might be published. Against this the actual and estimated expenditure is as follows :—

1.	Volume XXV, No. 4, April 1938 ..	Rs. —549 (due to receipts) (credited)
2.	Volume XXVI, No. 1, July 1938 ..	Rs. 4,865 (actual)
3.	Volume XXVI, Nos. 2 & 3, October 1938 ..	Rs. 6,500 (estimated)
	and January 1939 at Rs. 3,250 ..	Rs. 6,500 (estimated)
4.	Cost of *Memoir* No. 30	Rs. 2,849 (actual)
	Total	Rs. 13,665 (estimated)

A saving of approximately Rs. 1,835 may be anticipated.

J. *General.*—Publication of the *Journal* has been continued on the lines laid down at the Conference of 1932 and the services of the members of the Editorial Board have been made full use of by referring to them all papers dealing with the subjects in regard to which they have been nominated.

The lines of special research activity are reflected in the nature of papers received. In Vol. XXV out of 91 articles published 34 were on nutrition, 20 on biochemistry, 8 on haematology, 7 on medical zoology, and 6 on epidemic dropsy. In Vol. XXVI so far out of 73 articles 23 were on nutrition, 12 on biochemistry, and 8 on cholera.

Publications on medical research under the auspices of the Indian Research Fund Association during the year 1938.

A. Indian Journal of Medical Research and Indian Medical Research Memoirs.

AHMAD, B., and GORE H. K.—A diet survey of fifty-one families of Ferozepore, Punjab (Vol. 26, No. 1).

AHMAD, B., LAL, R. B., and ROY N. C.—Observations on the basal metabolism of healthy subjects under varying conditions of temperature and humidity (Vol. 26, No. 1).

AYKROYD, W. R., MADHAVA, K. B., and RAJAGOPAL, K.—The detection of malnutrition by measurements of arm, chest, and hip (Vol. 26, No. 1).

BASU, K. P., BOSE S. P., QUADER, M. A., and DE H. N.—Available carbohydrate and acid-base balance in pulses (Vol. 26, No. 3).

BASU, K. P., and DE, H. N.—Nutritional investigation of some species of Bengal fish. Part I. Biological value of the proteins of ruhee (*Labeo rohita*) and hilsa (*Clupea ilisa*) by the nitrogen balance and growth methods, and supplementary effect of fish on pulse proteins (Vol. 26, No. 1).

BASU K. P., and DE, H. N.—Nutritional investigation of some species of Bengal fish. Part II. Extraction and chemical analysis of the proteins of ruhee (*Labeo rohita*) and hilsa (*Clupea ilisa*) (Vol. 26, No. 1).

BASU, K. P., and DE, H. N.—Nutritional investigation of some species of Bengal fish. Part III. Determination of the vitamin-A content of the liver and body oils of ruhee (*Labeo rohita*) and hilsa (*Clupea ilisa*) by biological methods (Vol. 26, No. I).

CHOPRA, R. N., CHATERJEE, R. G., DE, N., and GHOSH, S.—A preliminary note on the chemistry and pharmacology of the leaves of *Skimmia laureola*, Hook F. (Vol. 26, No. 2).

CHOPRA, R. N., DAS, N. N., and MUKERJI, B.—The action of certain cardiac drugs on embryonic heart explants (Vol. 26, No. 1).

CHOPRA, R. N., and GANGULY, S. C.—Abstinence symptoms in opium addiction and the role of glucose in their treatment (Vol. 26, No. 3).

CHOPRA, R. N., GHOSH, S., and DUTT, A. T.—Some inorganic preparations of the Indian indigenous medicine. Part VI. *Samudra Phena* (Vol. 26, No. 2).

CHOPRA, R. N., MUKERJI B. and CHAKRAVARTY, M.—Studies on some dextro-rotatory hydrocupriendine derivatives. Part I. Comperative haemolytic activity (Vol. 26, No. 1).

CHOPRA, R. N., and ROY, A. C.—The effect of Seitz filtration on haemolysins and the components of a haemolytic Sera (Vol. 26, No. 1).

DE, N. K., and MAJUMDAR, B. N.—Further studies on factors affecting the vitamin-A activity of animal and vegetable Products (Vol. 25, No. 4).

DE, N. K., and MAJUMDAR, B. N.—The assimilation of carotene by rats from a 'poor madrassi Diet' : The influence of skimmed milk and calcium lactate (Vol. 26, No. 2).

DE, N. K., MAJUMDAR, B. N., and SUNDARARAJAN, A. R.—The vitamin-A activity of some fish oils and vegetable foods. Parts I and II (Vol. 26, No. 2).

DHARMENDRA and JOHN LOWE.—Attempts to cultivate *M.leprae muris* (Vol. 25, No. 4).

GHOSH, B. N., DE, S. S., and BHATTACHARYA, P. K.—Investigation on the isolation of the active principles from the venoms of *Bungarus fasciatus* and *Vipera russellii* (Vol. 26, No. 3).

GHOSH, B. N., and RAY, N. N. — Investigation on the occurrence of danysz phenomenon in diphtheria toxin-antitoxin mixtures under a variety of conditions (Vol. 26, No. 3).

GOYAL, R. K.—A serological study of the actinomyces (Vol. 25, No. 4)

GOYLE, A. N.—Observations on haemolysis by vibrios (Vol. 26, No. 3).

GREVAL, S. D. S., JOHN LOWE and BOSE, R. — Complement-fixation in leprosy with Witebsky, Klingenstein and Kuhn (W. K. K.) Antigen : A new technique (Vol. 26, No. 3).

JOHN LOWE, and GREVAL, S. D. S.— Complement-fixation in leprosy and other diseases by the Witebsky, Klingenstein and Kuhn (W. K. K.) Antigen (Vol. 26, No. 3).

KRISHNAN, B. G.—Calorie requirements of South Indian children (Vol. 26, No. 3).

LAL, R. B., and ROY, S. C.—Investigations into the epidemiology of epidemic dropsy. Part VI. Allylisothiocyanate as an aetiological factor in epidemic dropsy (Vol. 26, No. 1).

LAL, R. B., GHOSAL, S. C., and MUKERJI, B.—Investigations on the variation of the vibrios in the house fly (Vol. 26, No. 3).

LINTON, R. W., MITRA, B. N., and SEAL, S. C.—Electrophoresis and metabolism of some vibrio strains in relation to variability and chemical classification (Vol. 26, No. 2).

LINTON, R. W., SHRIVASTAVA, D. L., SEAL, S. C., and MOOKERJI, S. P.—Studies on the specific polysaccharides of the vibrios. Part II. chemistry and serology (Vol. 26, No. 1).

MUKERJI, B., and GUHA, B. C.—The effects of anterior pituitary extracts and choline on the liver-fat of rabbits (Vol. 26, No. 1).

NANDI, B. K., and DIKSHIT, B. B.—A new calorimetric test for plasmoquine (Vol. 25, No. 4).

NAPIER, L. E., and MAJUMDAR, D. N.—Haematological studies in Indians. Part IX. The analysis of the haematological findings in 57 cases of anaemia in pregnant tea-garden coolie women, with special reference to the results of treatment (Vol. 26, No. 2).

NARASIMHAMURTHY, G.—The nitrogen complex of Indian foodstuffs. Condiments : Part II. Chillies (*Capsicum annum*) and coriander seeds (*Coriandrum sativum*) (Vol. 25, No. 4).

PAL, R. K., and SINGH, NARINDRA—Further studies on the effect in rats of supplementing a South Indian diet with calcium and phosphorus (Vol. 26, No. 1).

PANDIT, S. R. and MOITRA, N. M.—Vibrios in natural water sources in Assam (Vol. 26, No. 1).

PASRICHA, C. L., CHATTERJEE, D. N., and DAS, P. C.—The distribution and characteristics of vibrios isolated from certain non-human sources in Calcutta (Vol. 26, No. 1).

PATWARDHAN, V. N., and CHITRE, R. G.—Studies in calcium and phosphorus metabolism. Part II. The effect of toxic doses of vitamin D on the Ash, Ca, and P Content of the bones and on the excretion of N, Ca, and P in albino rats (Vol. 26, No. 2).

RAJA, K. C. K. E., SATYA SWAROOP, BASAK, K. C., and LAL, R. B. Application of analysis of variance to mortality rates (Vol. 26, No. 1).

RAJAGOPAL, K.—The basal metabolism of Indian and European men on the Nilgiri Hills (S. India) (Vol. 26, No. 2).

RANGANATHAN, S.—Variations in the iron content of foodstuffs and the problem of iron requirements (Vol. 26, No. 1).

RAO, M. NARASIMHA.—Vitamin C and peptic ulcer (Vol. 26, No. 1).

RAO, M. V. RADHAKRISHNA.—Haematological investigations in South India. Part IV. Haemoglobin in normal pregnancy (Vol. 25, No. 4).

RAO, M. V. RADHAKRISHNA.—The study of myelin degeneration in polarized light (Vol. 26, No. 1).

ROY, A. C.—Lipolytic activity of the venoms (Cobra and Russell's Viper) (Vol. 26, No. 1).

ROY, A. C., and CHOPRA, R. N.—Some biochemical characteristics of snake venom (Vol. 26, No. 1).

SANKARAN, G., and RAJAGOPAL, K.—Haematological investigations in South India. Part V. The effect of altitude on haemoglobin content (Vol. 25, No. 4).

SANKARAN, G., and RAO, M. V. RADHAKRISHNA.—Haematological investigations in South India. Part III. The mean red cell diameter (Vol. 25, No. 4).

SEAL, S. C., and MITRA, B. N.—Oxidation-reduction potentials of *Vibrio cholerae* and related organisms (Vol. 26, No. 3).

SHORTT, H. E., HAWLEY, H., and SWAMINATH, C. S.—Iron-haematoxylin staining technique : An illusion (Vol. 26, No. 1).

SHORTT, H. E., PANDIT, C. G., and RAO, R. SANJIVA.—The virus of sandfly fever in culture and certain of its properties (Vol. 26, No. 1).

SWAMINATHAN, M.—The relative amounts of the protein and non-protein nitrogenous constituents occurring in foodstuffs and their significance in the determination of the digestibility Co-efficient of proteins (Vol. 25, No. 4).

SWAMINATHAN, M.—The relative value of the proteins of certain foodstuffs in nutrition. Part V. Supplementary values of the proteins of *Eleusine coracana* (Ragi) and of certain pulses and skimmed milk powder studied by the nitrogen balance and the growth method (Vol. 26, No. 1).

SWAMINATHAN, M.—The relative value of the proteins of certain foodstuffs in nutrition. Part VI. General conclusions (Vol. 26, No. 1).

SWAMINATHAN, M.—A chemical method for the estimation of nicotinic acid in biological materials (Vol. 26, No. 2).

TAYLOR, J., and AHUJA, M. L.—Incidence and characters of vibrios in waters in Northern India (Vol. 26, No. 1).

VISWA NATH, and GREWAL, K. S.—Cancer in India (Vol. 26, No. 3.)

WILSON, H. E. C., and MITRA, D. D.—A diet and physique survey in Assam, Rural Bengal, and Calcutta (Vol. 25, No. 4).

WILSON, H. E. C., and ROY, G. K.—The flavine and vitamin-B_6 (Anti-dermatitis) content of Indian foodstuffs (Vol. 25, No. 4).

WILSON, H. E. C., and ROY, N. C.—Observations on the basal metabolism of Indian boys in Calcutta (Vol. 25, No. 4).

B. *Journal of the Malaria Institute of India.*

AFRIDI, M. K.—Observations on extra-abdominal spleen in monkeys infected with *P. cynomolgi* and *P. knowlesi.* (Vol. I, No. 4).

AFRIDI, M. K. and MAJID, S. A.—Observations on the range of dispersion of *Culex fatigans*, and its infiltration into the Delhi urban area. (Vol. I, No. 2).

AFRIDI, M. K., and MAJID, S. A.—Malaria in Bahrein Islands. (Vol. I, No. 4).

AFRIDI, M. K., MAJID, S. A., and SINGH, J.—Malaria in Kutch State. (Vol. I, No. 2).

BASU, B. C.—Studies on a malarial infection in a paddy bird. (Vol. I, No. 3).

BUTT, N. M.—Inexpensive apparatus for mixing larvicides. (Vol. I, No. 2).

CHOPRA, R. N., and BASU, B. C.—Studies on the effect of anti-malarial drugs upon the infectivity of patients to mosquitos : Pt. II, 'Cilional'. (Vol. I, No. 4).

COVELL, G.—Malaria Survey of India, 1927-37. (Vol. I, No. 1).

COVELL, G., MULLIGAN, H. W. and AFRIDI, M. K.—An attempt to control malaria by the destruction of adult mosquitos with insecticidal sprays. (Vol. I, No. 1).

C. *Lists of papers published by workers under the I.R.F.A. in Journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

AYKROYD, W. R. and KRISHNAN, B. G.—Effect of calcium lactate on children in a nursery school. *The Lancet.* July 16th, 1938. p. 153.

BASU, K. P. and NATH, M. C.—Organic acid -Fe complex as a disturbing factor in the titrimetric estimation of ascorbic acid. (Journal Indian Chemical Society, Vol. XV. Issue No. 3).

CHATTERJEE, H. N.—"Studies in the pathology of haemo-lymph organs in cholera". Paper presented before the 26th session Indian Science Congress.

CHATTERJEE, H. N.—"Clinical aspects of anaemia of pregnancy and their relation to haematology." I. M. G., May 1938; Vol. LXXIII, P. 267.

CHATTERJEE, H. N.—"Erythrocytic studies in the anaemia of pregnancy as a guide to the blood pathology and therapy." Jour. Ind. Med. Assen. October, 1938; Vol. IX, P. 1.

CHATTERJEE, H. N.—"Studies in the dimensions of erythrocytes of man." Published by the Calcutta University. University Publication Series 1938, Calcutta.

CHATTERJEE, H. N. and DAS GUPTA, B. M.—"Observations on a bodo-like flagellate persistently occurring in the faeces of man." Parasitology, Jan, 1938; Vol. XXX, P. 56.

CHATTERJEE, H. N. and GHOSH, S. N.—"Further researches in biochemistry of pregnancy anaemia and of normal pregnancies in Bengal". Pape presented befo re the 26th session Indian Science Congress.

CHATTERJEE, D. N. and MALIK, K. S.—The bacteriological examination and the hydrogen-ion concentration of the urine of a series of 122 cholera patients. I.M.G., Vol. LXXIII, No. 10.

CHOPRA, R. N., and DAS GUPTA, B. M.—A note on the therapeutic efficiency of solu-septasin in simian malaria (*P. knowlesi*). *I.M.G.*, Vol. LXXIII, 1938.

CHOPRA, R. N., and DAS GUPTA, B. M.—Studies on the action of synthetic anti-malaria drugs on the treatment of Indian strains of malaria. 'Cilional' in the treatment of crescent carriers. *I.M.G.*, Vol. LXXIII, 1938.

CHOPRA, R. N., and DAS GUPTA, B. M.—Action of prontosil in monkey malaria. *I.M.G.*, Vol. LXXIII, 1938.

CHOPRA, R. N., and CHOPRA, G. S.—The opium smoking habit in India and present position of the opium smoking habit in India, Part I. Indian Med. Gaz., Vol. LXXIII, No. 2.
The opium smoking habit in India and present position of the opium smoking habit in India, Part II. *I.M.G.*, Vol. LXXIII, No. 3.
The opium smoking habit in India and present position of the opium smoking habit in India, Part III. *I.M.G.*, Vol. LXXIII, No. 4.

CHOPRA, R. N., and GHOSH, N. N.—Aloe vera, Linn. Aloe indica, Roxb. I. M. G. 276, 348-50.

CHOPRA, R. N., and GHOSH, N. N.—Erythroxylon monogynum Roxb. I. M. G. 276, 340-3.

CHOPRA, R. N., SUNDAR RAO, S., and MUKHERJEE, S.—The relative composition of blood and lymph in filarial infection. Proc. Ind. Sc. Congress, 1938, P. 47.

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DAS GUPTA, B. M.—Leptospirosis in India. *I.M.G.*, Vol. LXXIII, 1938.

DISKHIT, B. B.—Proc. Nat. Inst. of Sciences, India Vol. IV, No. 2.

DUTTA, A. T., CHATTERJEE, N. R., GHOSH, S., and CHOPRA, R. N.—Paris polyphylla Sm. Part I. Arch. Pharm. 276, 343-5.

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GHOSH, B.—Observation on the relation between pregnancy, sex-hormones and vitamin C content of the tissues of guinea-pigs. (J. Indian. Chem. Soc. Vol. 15, No. 8.)

GHOSH, B. N.—Enzyme in snake venom—Part IV. Jour. Ind. Chem. Soc., 15, 1938, p. 571.
Destruction of the neurotoxin of cobra (Naja Naja) and daboia (Vipera Russellii) venom by various reducing agents. Science and Culture, 4, 1938, p. 198.

The determination of isœlectric point of the neurotoxin of the Russell's viper venom. Science and Culture, 3, 1937, p. 297.

GHOSH, N. N., GHOSH, S, and CHOPRA, R. N.—Bambusa arundinacea Wild. *I.M.G.*, Vol. LXXIII, 276, 351-3.

GHOSH, B., and GUHA, B. C.—Vitamin C and toxins Part I. (J. Indian. Chem. Soc. Vol. 15. No. 8.)

GHOSH, B., and GUHA, B. C.—Vitamin C and Toxins Part II. (J. Indian Chem. Soc. Vol. 15. No. 8.)

GUHA, B. C., and SEN GUPTA, P. N.—Ascorbigen in plant and animal tissues.

(Nature 1938, Vol. 141, p. 974).

LAHIRI, M. N., and DAS, P. C.—Vibrios from certain non-human sources. *I.M.G.*, Vol. LXXIII, No. 11.

LAHIRI, J. K., GHOSH, S., and CHOPRA, R. N.—Thevatia neriifolia, Juss. *I.M.G.*, Vol. LXXIII, 276, 345-7.

LOWE, J.—Preliminary report of an epidemiological survey of leprosy in a typical rural area of West Bengal. ("Leprosy in India" Vol. X, No. 2).

LOWE, J.—The leprosy problem in Burma ("Leprosy in India Vol. X, No. 4).

LOWE, J.—A note on racial variations in leprosy with particular reference to Indians and Burmese races. (*I.M.G.* Vol. LXXIII, No. 10).

LOWE, J.—*Book*: Lecture notes on leprosy.

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("Leprosy in India" Vol. X, No. 1)

LOWE, J., and CHATTERJEE, S. N.—Extensive ulceration of the skin in leprosy.
("Leprosy in India, Vol. X, No. 1).

MAPLESTONE, P. A., and SUNDAR RAO, S.—Dracontiasis—a pamphlet. (Public Health Bulletin).

MAPLESTONE, P. A., and SUNDAR RAO, S.—Tail of male *Wuchereria bancrofti*. (accepted by Records of Indian Museum).

NAPIER, L. E., DAS GUPTA, C. R., SEN, G. N., RAI CHAUDHURI, M. N., SEN GUPTA, P. C., and MAJUMDAR, D. N.—Anaheamin in tropical macrocytic anaemia. *I.M.G.*, Vol. LXXIII, No. 7, July, 1938.

PASRICHA, C. L., CHATTERJEE, D. N., and MALIK, K. S.—The hydrogen-ion concentration of cholera stools. *I.M.G.*, Vol.LXXIII, No. 6.

PASRICHA, C. L., CHATTERJEE, D. N., and PAUL, B. M.—Studies on the potency of prophylactic vaccines. 1. Cholera vaccine. *I.M.G.* Vol. LXXIII, No. 8.

PASRICHA, C. L., DEMONTE, A. J. H., and CHATTERJEE, B. C.—*Vibrio cholerae* from material obtained by liver puncture during life. *I.M.G.* Vol. LXXIII, No. 7.

PASRICHA, C. L., LAHIRI, M. N., and DAS, P. C.—The isolation of *vibrio cholerae* from non-cholera individuals. *I.M.G.*, Vol. LXXIII, No. 11.

RANGANATHAN, S., and NARASIMHAMURTY, G.—The chemical composition of buttermilk. *Agriculture and Livestock in India.* July, 1938. 8 (4). p. 421.

RANGANATHAN, S., and NARASIMHAMURTI, G.—Foods and nutrition. *Annual Review of Biochem and Allied Research in India.* 1937. 8. pp. 68-88.

RAO, M. V. RADHAKRISHNA.—The treatment of phrynoderma by vitamin A concentrate. Ind. Med. Gaz. LXXIII (8). p. 461.

RAY, P. N., and SUNDAR RAO, S.—Chyluria of filarial origin. (accepted by Br. Jl. Urology).

SEN, P. K.—Marriage and tuberculosis. (Journ. Tuberc. Assocn. Bengal, June, 1938).

SEN, P. K.—Industry and tuberculosis. ("Science and Culture", Vol. IV, No. 2, Aug. 1938).

SEN, P. K.—After-care of tuberculous patients. (Trans. Med. Coll. Re-Union, Vol. I, No. 1, pp. 106-110, 1938).

SEN GUPTA, P. N., and GUHA, B. C.—The estimation of vitamin C in foodstuffs. (Science and Culture, Vol. 3. No. 7).

SMITH, R. O. A., MACGUIRE, J. P., STEPHENS, E. D., LAHIRI, B. N.—"The treatment of animals with antirabic vaccine" (In press. The Indian Medical Gazette).

SUNDAR RAO, S.—Treatment of Filarial Lymphangitis. Proc. Ind. Sc. Congress, 1938, p. 232.
Effect of malarial Toxin on filarial infection. Ibid. p. 232.
Distribution of filarial infection in Calcutta. Ibid. p. 240.
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SUNDAR RAO, S.—Filarial infection and filarial diseases. Health number, Calcutta Municipal Gaz. April, 1938.

SUNDER RAO, S.—Recent researches on surgical complications in Filariasis. Science and Culture, Vol. IV, p. 183.

SWAMINATHAN, M.— A calorimetric method for the estimation of nicotinic acid in foodstuffs.
Nature. May 7th, 1938. 141(3575), p. 830.

UKIL, A. C.—Some points in the diagnosis of pulmonary tuberculosis (adult type).
(Trans. Med. Coll. Re-Union, Vol. 1, No. 1, 1938, pp. 95-99).

UKIL, A. C.—Anti-tuberculosis work in Bengal (Ind. Med. Gaz., Sept., 1938).

UKIL, A. C.—Tuberculosis in India.
(Office International d'Hygiene Publique, Tome XXX, No. 9, Sept., 1938, pp. 2066).

UKIL, A. C.—Tuberculosis among "home contacts" (*Ibid*, p. 250).

UKIL, A. C., and DE, K. N.—The applicability of collapse therapy at the out-patients' section of a city hospital.
(*Ibid*, pp. 233).

UKIL, A. C., and GUHA THAKURTA, S. R.—The influence of sunlight upon experimental tuberculosis.
(Amer. Rev. Tuberc., April, 1938).

UKIL, A. C., and GUHA THAKURTA, S. R.—A tuberculin survey in a hill area in the Darjeeling district.
(Proc. Ind. Sc. Congress 1938, pp. 250).

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on 17th December, 1938.

	Enquiries	Budget allotments
1.	Cholera field enquiry in Bengal by Major W. D. B. Read, I.M.S.	Rs. 33,000
2.	Cholera enquiry under the Director, King Institute, Guindy, Madras	Rs. 8,020
3.	Cholera statistical enquiry by Dr. R. B. Lal at the All India Institute of Hygiene and Public Health, Calcutta	Rs. 2,620
4.	Cholera vibrios enquiry under the Director, Central Research Institute, Kasauli ..	Rs. 3,125
5.	Cholera bacteriological enquiry under Major C. L. Pasricha, I.M.S. at the School of Tropical Medicine, Calcutta	Rs. 6,120
6.	Enquiry on cholera toxins and immunity by Dr. H. Ghosh at the Indian Institute of Medical Research, Calcutta	Rs. 3,800
7.	Malaria Institute of India	Rs. 1,02,397
8.	Malaria transmission enquiry under Dr. B. M. Das Gupta at the School of Tropical Medicine, Calcutta	Rs. 6,960
9.	Blackwater fever enquiry by Dr. K. V. Krishnan at the All India Institute of Hygiene and Public Health, Calcutta	Rs. 5,368
10.	Enquiry on the maxillary index of A. stephensi in Calcutta by Mr. Senior White, Malariologist, B. N. Railway, Calcutta	Rs. 412
11.	Nutrition researches under Dr. W. R. Aykroyd at Coonoor	Rs. 85,925
12.	Enquiry on cheap well balanced diets under the Bombay Presidency Baby and Health Week Association	Rs. 7,597
13.	Investigation on basal metabolism in children and adults in Bombay Presidency under Dr. S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay	Rs. 2,429
14.	Ascorbic acid enquiry by Dr. B. C. Guha at the University College of Science and Technology, Calcutta	Rs. 5,100
15.	Enquiry on food analysis by Dr. K. P. Basu, Dacca University, Dacca..	Rs. 4,740
16.	Researches on working efficiency of Indian labourers under Professor W. Burridge at the King George's Medical College, Lucknow ..	Rs. 2,000

	Enquiries	Budget allotments
17.	Enquiry into malnutrition under Professor N. M. Basu at the Presidency College, Calcutta ..	Rs. 2,500
18.	Investigation into the level of blood phosphorus and serum calcium, etc. by Dr. D. C. Wilson ..	Rs. 600
19.	Enquiry for detection of adulteration of food-stuffs by Rai Sahib Dr. N. K. Ghosh, Public Analyst, Assam, Shillong	Rs. 1,500
20.	Nutritional investigation on Bengal fish by Dr. B. C. Guha at the University College of Science and Technology, Calcutta	Rs. 3,040
21.	Investigation on calcium metabolism in health and disease by Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay ..	Rs. 2,700
22.	Additional nutrition enquiry under Dr. W. R. Aykroyd. (*The cost of this enquiry to be met from the Rs.* 30,000 *special annual grant sanctioned by the Governing Body over a period of five years, out of the special ten lakhs grant made by Government for anti-malaria work*). ..	Rs. 27,327
23.	Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta ..	Rs. 5,168,
24.	Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leper Settlement, Chingleput Madras	Rs. 2,460
25.	Plague researches under the Director, Haffkine Institute, Bombay	Rs. 32,109
26.	Enquiry into the recrudescence of plague in the Cumbum Valley by Dr. P. V. George, Research Health Officer, Madras	Rs. 1,500
27.	Anti-rabic vaccine enquiry under the Director, Pasteur Institute of India, Kasauli ..	Rs. 1,500
28.	Tuberculosis enquiry under Dr. A. C. Ukil at the All India Institute of Hygiene and Public Health, Calcutta	Rs. 12,788
29.	Tuberculosis enquiry by Major G. F. Taylor, I.M.S., at the K. E. Medical College, Lahore ..	Rs. 500
30.	Indigenous drugs enquiry under Bt.-Colonel R. N. Chopra, I.M.S., at the School of Tropical Medicine, Calcutta	Rs. 24,071
31.	Indigenous drugs enquiry by Dr. Khem Singh Grewal at the K. E. Medical College, Lahore ..	Rs. 2,041
32.	Analysis of the material collected by the enquiry into causes of anaemia in women at the All India Institute of Hygiene and Public Health, Calcutta	Rs. 500

	Enquiries	Budget allotments	
33.	Enquiry into anaemia of women in Assam under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta	Rs.	5,296
34.	Enquiry into toxaemia of pregnancy by Dr. N. A. Purandare at the Seth G. S. Medical College, Bombay	Rs.	3,398
35.	Rural statistical enquiry into maternal mortality in and around Delhi by Dr. M. I. Neal Edwards, Acting Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi	Rs.	3,350
36.	Investigation of biochemical and dietetic factors concerned in the toxaemia of pregnancy by Dr. Vishwa Nath at the K. E. Medical College, Lahore	Rs.	1,200
37.	Helminthological enquiry under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta	Rs.	3,648
38.	Enquiry into medical mycology by Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta	Rs.	6,978
39.	Dracontiasis and filariasis enquiry under Dr. S. Sundar Rao at the School of Tropical Medicine, Calcutta	Rs.	4,776
40.	Protozoal parasites enquiry under the Director, Pasteur Institute of Southern, India, Coonoor	Rs.	9,500
41.	Collection of further data regarding cancer by Dr. Vishwa Nath of the K. E. Medical College, Lahore	Rs.	700
42.	Typhus enquiry under the Director, Pasteur Institute of India, Kasauli	Rs.	7,732
43.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras ..	Rs.	500
44.	Epidemic dorpsy enquiry (laboratory section) under Major C. L. Pasricha, I.M.S., at the School of Tropical Medicine, Calcutta	Rs.	2,924
45.	Epidemic drposy enquiry (field section) under Dr. R. B. Lal at the All India Institute of Hygiene and Public Health, Calcutta ..	Rs.	5,426
46.	Kala-azar enquiry by Dr. L. E. Napier at the School of Tropical Medicine, Calcutta ..	Rs.	4,472
47.	Kala-azar enquiry in Bihar by Dr. R. O. A. Smith	Rs.	15,528
48.	Toxicological medico-legal investigation by Rai Bahadur Dr. K. N. Bagchi, Chemical Examiner to the Government of Bengal, Calcutta ..	Rs.	1,000

	Enquiries	Budget allotments	
49.	Enquiry on bone marrow and secondary anaemias by Dr. H. N. Chatterjee at the Carmichael Medical College, Calcutta	Rs.	1,888
50.	Enquiry into thrombo-angeitis obliterans by Dr. R. N. Chopra at the Seth G. S. Medical College, Bombay	Rs.	2,200
51.	Pharmacological enquiry by Dr. B. B. Dikshit at the Haffkine Institute, Bombay	Rs.	9,500
52.	Enquiry on algae present in water supply reservoirs and their effect on purification methods and on health under the Director, King Institute, Guindy, Madras	Rs.	2,620
53.	Sandfly fever enquiry under the Director, King Institute, Guindy, Madras	Rs.	6,344
54.	Enquiry into endemic fluorosis under the Director, King Institute, Guindy, Madras ..	Rs.	3,944
55.	Enquiry into isolation of the different constituents of snake venoms, etc. by Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta	Rs.	2,800
56.	Investigation into the etiology of infantile biliary cirrhosis with special reference to its anaemia by Dr. K. Narayana Murthi at the Medical College, Madras..	Rs.	3,270
57.	Researches on flies by Dr. C. Strickland at the School of Tropical Medicine, Calcutta ..	Rs.	2,500
58.	Tissue culture enquiry by Dr. G. Sankaran at the All India Institute of Hygiene and Public Health, Calcutta	Rs.	3,220
59.	Assessment of malnutrition of Bengali boys with vitamin 'A' deficiency by Professor N. M. Basu, Presidency College, Calcutta ..	Rs.	2,400
60.	Investigation into the problem of dental caries in India by Dr. Rahman Siddiqi, School Health Officer in the United Provinces	Rs.	2,000
61.	Research on cholera kidney by Dr. D. N. Bannerjee, Carmichael Medical College, Calcutta	Rs.	2,500
62.	Investigation of indigenous drugs by Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay	Rs.	2,210
63.	Enquiry into filariasis by Dr. T. Bhaskara Menon, Professor of Pathology, Medical College, Vizagapatam	Rs.	2,540
64.	Anthropological study of human brains by Professor of Anatomy, Medical College, Vizagapatam	Rs.	100

	Grants		Budget allotments
(*i*)	Administration charges	Rs.	29,104
(*ii*)	Contribution towards the cost of two chairs at the School of Tropical Medicine, Calcutta ..	Rs.	16,770
(*iii*)	Contribution to the Imperial Institute of Entomology, London	Rs.	1,350
(*iv*)	Contribution to the Bureau of Hygiene and Tropical Diseases, London	Rs.	2,025
(*v*)	Contribution towards the cost of the Statistical Bureau under the Public Health Commissioner with the Government of India	Rs.	5,200
(*vi*)	Indian Journal of Medical Research and Memoirs		
	(*a*) Publication charges of the Journal .. Rs. 13,000		
	(*b*) Publication charges of Memoirs Rs. 2,500		
	(*c*) Editor's office .. Rs. 3,924	Rs.	19,424
(*vii*)	I.R.F.A. Library at the Central Research Institute, Kasauli	Rs.	2,140
(*viii*)	Pay and contributions of the officers of the Medical Research Department under the Association	Rs.	1,50,000
(*ix*)	House rent, compensatory and travelling allowances and certain contingent expenses payable to the eight officers of the Medical Research Department	Rs.	7,500
(*x*)	Pay of three junior research workers ..	Rs.	17,385
(*xi*)	Pay of two clerks at the School of Tropical Medicine, Calcutta	Rs.	2,640
(*xii*)	Clerical assistance to the Director, All India Institute of Hygiene and Public Health, Calcutta	Rs.	1,020
(*xiii*)	Travelling allowances of subordinate workers on transfer from one post to another ..	Rs.	1,000
(*xiv*)	I.R.F.A. contribution and interest in the event of a Contributory Provident Fund for non-Government servants if instituted from 1939-40	Rs.	12,000

REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1939

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The Scientific Advisory Board consisted of the following :—

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Major-General E.W.C. BRADFIELD, C.I.E., O.B.E., K.H.S., I.M.S., Director-General, Indian Medical Service. (1st January to 29th September, 1939).

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Lieut.-Colonel G. COVELL, C.I.E., I.M.S., Director, Malaria Institute of India, Kasauli. (1st January to 5th August and 29th September to 31st December, 1939).

Major M. K. AFRIDI, I.M.S., Offg. Director, Malaria Institute of India, Kasauli. (6th August to 28th September, 1939).

Dr. R. ROW, O.B.E., 27, New Marine Lines, Fort, Bombay.

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Dr. VISHWA NATH, Assistant Professor of Pathology, King Edward Medical College, Lahore.

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Dr. W. R. AYKROYD, Director, Nutrition Research, I. R. F. A., Coonoor.

Dr. M. I. NEAL EDWARDS, W.M.S., Director, Maternity and Child Welfare Bureau, Indian Red Cross Society, New Delhi.

Dr. FRIMODT MÖLLER, Medical Commissioner, Tuberculosis Association of India, New Delhi.

Dr. DHARMENDRA, Leprosy Research Worker, School of Tropical Medicine, Calcutta.

Composition of Advisory Committees for the year 1940.

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Lt.-Col. P. B. BHARUCHA.

The Board held two meetings during the year on the 18th and 20th December 1939.

At its meeting on the 18th December, 1939, the Board confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers, and also decided on the cooption of experts in special subjects for their meeting on the 20th December, 1939. On the 20th December, 1939, the Board considered in detail the programme of research for the year 1940-41. The programme of research recommended by the Board is given on pages 185—188 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1939.

The researches, which were carried out during the year under report, and which were recommended by the 16th Medical Research Conference held at New Delhi from the 12th to 16th December, 1938, were finally approved by the Governing Body of the Association at their meeting held in the Imperial Secretariat, New Delhi, on the 4th March, 1939.

CHOLERA.

1. Cholera field enquiry under Dr. S. R. Pandit and Major W. D. B. Read, I.M.S., at Khulna, Bengal.

During the first two months of this enquiry work was carried out at the All-India Institute of Hygiene and Public Health in Calcutta, where equipment was collected and personnel trained in the methods to be adopted in the field. During this period a survey was made of part of the district of Khulna, which it was thought would prove suitable for the work. A laboratory site was selected at Khulna town and two collecting stations further down the delta, as it was not possible to secure suitable accommodation for the laboratory outside the headquarter town of the district.

Selection of the site for work.

The district of Khulna was selected for the following reasons :—

(1) It is situated in a district where the geographical and other factors peculiar to the delta are well marked. It was thought that the factors responsible for the endemicity of cholera in Bengal might be connected with its deltaic character. Other areas in which cholera incidence approximates to the type in Bengal, *e.g.* Tanjore, Puri and Cuttack, are also deltaic.

(2) Absence of contiguity with Calcutta was thought likely to remove, as far as possible, the influence of factors due to repeated reinfections from outside the area, which cannot be excluded from results obtained in districts adjacent to Calcutta.

(3) It has a high general cholera incidence.

(4) The monsoon decrease of cholera incidence which is so marked a feature of the Bengal type of cholera is well marked in Khulna district.

(5) The presence of salt water which exists almost everywhere in the southern half of the district was regarded as an indispensable condition for selection in view of the importance which has been attached on experimental and epidemiological grounds to this factor.

" (6) The water supplies in the area were not supervised by health authorities.

(7) Annual migrations on the part of the population are at a minimum.

Two collecting stations were selected as it appeared, at the time of the preliminary survey, that one was a relatively sweet water type and the other salt water. This distinction was found not to hold throughout the period of investigation but by good luck another basis for differentiation was discovered, the value of which it is at present too early to try to assess.

General aim of the work done.

It was realised that it was quite impossible to do anything in the nature of a general survey of the population owing to the fact that the staff was insufficient for the purpose, to which may be added the additional complicating factor that cholera is widely scattered throughout the district and very few cases can be expected to occur in any one area. It was therefore decided to depend on a detailed examination of a few samples and to make the sampling as representative as possible. A rather different policy with regard to collection of samples was adopted in the two areas. In the salt water area there was a large number of repeats of the water samples due (a) to the fact that it was decided to restrict the area examined to narrow limits and (b) to the decision to repeat those samples which were positive to discover, if possible, the length of persistence of the vibrios in any one given source. In the salt water station this meant that there were few if any repeat samples of stools but many of water. In the sweet water station on the other hand a move was made from one village to another with the result that there were few repeat samples of either stools or water, with the exception of those done at the time of a small outbreak of cholera which was investigated.

Results obtained.

Stool specimens. Between the 20th June and 30th November, a total of 2,541 stools was examined and a careful record of the particulars of the individuals who passed them was kept. Except at the time of the solitary cholera outbreak with which the enquiry was able to make contact during the period of investigation, there were no agglutinable vibrios isolated from the stools. The sensitivity of the method employed, which was the modified Wilson and Blair method recently elaborated in India, and which was carefully controlled every week, led to the belief that this meant that the agglutinable vibrio was not present in the stool at the time of inoculation. There were various reasons which suggested that the technical method was not at fault. As evidence of this may be noted (a) the water results showed that the vibrios could be isolated with comparative ease and experimentally the method used for waters is inferior to that used for stools in the degree of ease with which the results can be regularly obtained; (b) in the small outbreak of cholera mentioned above there were six cases of clinical cholera and two of diarrhoea. All these were found to be positive for agglutinable vibrios. Also of 63 contacts passing normal faecal stools 5 were found to be positive. At the same time it was obvious not only in this epidemic but also from the general results that there was more evidence that the water supplies were infected than the stools of the users. While the examinations that could be carried out might justly be considered inadequate to decide the matter the suspicion arose that the vibrios while appearing in the tank waters were being killed off in the stool

samples before they could be submitted to the right conditions for survival and growth in the media bottles. This view was also supported by the finding that of the stools in the salt water area approximately 50% were at a pH of between 5 and 6 and 50% between 6 and 7. It is known that in pure culture in peptone water the vibrio will not multiply at a pH below 6 and is killed off slowly at 5.6, this from a fairly large inoculum likely to be much in excess of the number of vibrios that might occur in the stool of a "carrier". The exact conditions for survival in stool samples are not known but are not likely to be more favourable. It may therefore be concluded that the work so far carried out has not proved that the agglutinable vibrio is not present in the intestines of the local population in spite of the negative results. An attempt is being made to alkalinise the stools at the moment of collection, there being an interval of some two hours before the stool can be inserted into the enrichment bottle by the method used at present. This is, however, beset with technical difficulties for workers away from laboratory help.

In the cholera outbreak mentioned above all the stool samples except one contained the Ogawa non-haemolytic type—one specimen from a case of clinical cholera contained Ogawa haemolytic. This was from a small child which recovered and which had access to a water supply proved at the time of the epidemic and on two other occasions to contain the same El Tor type. There were also connected cases of cholera which gave the non-haemolytic type and the village in which the child lived was miles from the scene of the main outbreak investigated, though it was proved that there was connection between the two by the transference of one case. The scene of the main outbreak gave nothing but Ogawa non-haemolytic type.

Water specimens.

Bacteriological. In the salt water area 835 samples from 564 sources were examined and in the sweet water area 500 samples from 408 sources. (A source was taken as a sample which had not previously been examined in any given month. Many tanks were examined more than once in one month but would in that case be counted as 'one source'.) In the former area 43 samples and 35 sources were found positive for the agglutinable vibrio and in the latter 13 samples and sources were positive (apart from 10 strains isolated in the presence of cholera). This represents 5% from samples and 6% from sources in the former and 3% and 4% in the latter.

The monthly details are summarised :—

Salt water station.

Month	Samples	Positive	%	Sources	Positive	%
June	60	0	0	60	0	0
July	135	17	13	107	12	11
August	148	15	10	51	12	24
September	192	5	3	141	5	4
October	96	3	3	88	3	3
November	204	3	1	117	3	3

Sweet water station.

Month	Samples	Positive	%	Sources	Positive	%
June	42	0	0	42	0	0
July	106*	10*	9*	63*	8*	14*
August	105	4	4	84	4	5
September	96	2	2	83	2	2
October	48	3	6	33	3	9
November	103	4°	4°	103	4°	4°

*Includes vibrios isolated during the epidemic outbreak.
°May represent the result of epidemic infection. The results are not yet available.

From the above it will be seen that there was a rapid drop in the positive isolations of the salt water station some time during August or September (actually it was in the middle of August) while in the sweet water station they remained more or less at a steady level throughout.

The vibrios isolated represented 4 different types, Inaba and Ogawa, haemolytic and non-haemolytic. The Ogawa types were much commoner than the Inaba, especially the haemolytic Ogawa type. The actual numbers of each were as follows :—

Morrelganj :—

Inaba H	1
Inaba NH	2
Ogawa H	29
Ogawa NH	13
Undetermined	3

Bagherhat :—

Inaba H	Nil
Inaba NH	,,
Ogawa H	10
Ogawa NH (non-epidemic)	1
,, ,, (epidemic)	9
Undetermined	4

On several different occasions different types of vibrios both from the point of view of serology and haemolysis were isolated from the same tank both on the same and different days.

In the cholera outbreak all vibrios except one (mentioned above under stool specimens) were of the Ogawa non-haemolytic type.

Chemical. The specimens of water, which were examined bacteriologically, were also examined chemically for the following constituents :—

A. total solids %.
B. chlorides estimated as NaCl %.
C. oxygen absorption parts per 100,000.
D. presence of H_2S.
E. pH.

A small number of specimens was also submitted to a detailed analysis to secure a quantitative estimation of the basic and acid radicals present in the water.

Of the above only the total solids and chlorides were found to yield results of interest. The amount of oxydisable matter present and the pH in all specimens were adequate for multiplication. H_2S was absent. Estimations other than total solids %, and chlorides % are not being carried out now except on selected specimens.

The position with regard to the total solids and chlorides may be estimated from the table below.

	Salt water area.		Sweet water area.	
	Total Solids % (median average)	Ratio TS/Cl	TS % (median average)	Ratio TS/Cl
June	0.12	1.6	0.03	5.8
July	0.09	1.7	0.04	4.5
August	0.04	2.5	0.03	3.5
September	0.03	3.3	0.03	3.7
October	0.03	3.3	0.03	4.2
November	0.03	3.3	0.02	4.9

There were very few appreciable individual variations from the median average cited above.

The above table shows that in the sweet water station there was little or no variation while in the salt water station there was a considerable drop from July onwards. Actually the fall could be traced from day to day from the beginning of last week of July to the end of first week of August. By that time conditions in both areas were much the same and remained so up to the end of the period of examination. Coincident with the fall of total solids the total solids/chlorides ratio rose till similar figures were being recorded in both centres. A rise in the ratio of course indicates that the proportional amount of chloride is decreased.

River water analysis. In the salt water station on July 17th the total solids percentage was 0.09 and the ratio of total solids to chlorides was 1.5. By the 16th August these had become 0.01 and 2.1 and by the 4th September 0.02 and 3.7, at which figure they became stationary. In the sweet water station unfortunately no analysis of the river were carried out before September when the figures were '0.02% and 6.0'. Similar figures were recorded in October and November.

RAINFALL FIGURES.

	Salt water area.	Sweet water area.
May	1.39	4.80
June	7.20	11.95
July	26.12	27.20
August	18.64	15.70

(Figures for the succeeding months are not available.)

A short note on the significance of these findings is added after the epidemiological note.

Epidemiology.

As already recorded this enquiry has only been able at the time of writing to investigate one small outbreak of cholera. The majority of the water isolations were therefore made in the absence of cholera and no proof of their connection with cholera is yet forthcoming.

The following epidemiological table may, however, be noted in relation to the findings, bacteriological and chemical, recorded above.

The table gives the cholera deaths for the years 1901-1932 (median average) and for the present year.

	Salt water area.		Sweet water area.	
	1901-1932	1939	1901-1932	1939
April	67	36	44	9
May	23	3	34	4
June	14	1	16	12
July	2	0	6	19
August	0	0	5	0 (2 non-fatal cases occurred)
September	0	0	2	0
October	0	0	1	1
November	0	0	11	2
December	21	X	52	X

Note on the significance of the above findings.

The conditions in the sweet water area seem much more stabilised than in the salt water area. The percentage of water isolations changed about the middle of August in the salt water area but there was no change in the sweet water area. The chemical changes took place at the beginning of August and also in the salt water area only. Cholera disappeared from the salt water area in June but was present in the sweet water area up to July when there was a considerable outbreak. In other years it continued throughout the summer. On the other hand the average total incidence of cholera is higher in the salt water area than in the sweet water area.

The alteration in the tank waters is almost certainly due to changes in the character of the river waters in the salt water area. This has an easy explanation in the observation that nearly all the tanks in the salt water area are in direct connection with the river system which not only flows in and out of them with the tide but at flood periods overflows most of the country. The reverse holds in the sweet water area where there is no connection with the river system. Whether the other factors investigated *e.g.* cessation of cholera, increase in the rainfall, fall in the total solids percentage and rise in the total solids/chlorides ratio and the decrease in agglutinable vibrios can be linked also with changes in the river system remains to be seen, but their alterations follow parallel courses,

though with some time lag between them. From this point of view one collecting station forms a useful control to the other.

AGGLUTINATION TESTS ON SERA.

Blood sera of 167 individuals, 4 of whom were cholera contacts and the remaining normal individuals, were examined for cholera agglutinins. Eighteen *i.e.* 11%, gave agglutination in a dilution of 25 or over with Inaba or Ogawa or both, the suspensions being formolised agar washings. Titres beyond 50 were not obtained. Of the 18 positives, 2 agglutinated Inaba only, 7 agglutinated Ogawa only and 9 both. The sera of the 4 contacts were negative. Two individuals who gave a history of an attack of cholera 6 months previously and five who had cholera inoculation about a year or more previously were also negative.

In the examination of the earlier specimens, formalinised as well as boiled agar washings of Inaba, Ogawa and Inaba Rough were used, but it became evident that whereas with formalinised suspensions of Inaba and Ogawa very few sera were positive, a great majority of them agglutinated the boiled suspensions, and the question arose whether this had any significance. These boiled suspensions have been found to give reactions with almost all "O" sera of the various types of inagglutinable vibrios. This finding, taken along with the observed fact that a large majority of the sera examined gave positive reactions, suggests the necessity of further observations whether such agglutination can be taken as specific and as a measure of the "O" titre. The same remarks apply to formalinised and boiled agar washings of Inaba Rough, with which also 70% of sera were positive. Agglutination tests were put up in Felix tubes and readings of one plus and over were taken as positive.

Recently, suspensions heated at 70° for an hour and alcoholised suspensions have been employed. Comparatively few sera have been examined with these, but there have been many positives though the number is slightly less than with boiled suspensions.

2. Cholera field enquiry under the Director, King Institute, Guindy.

DISTRIBUTION OF THE TYPES OF *V. cholerae*.

At the beginning of the year there was a severe outbreak of cholera on the Cauvery delta. Thirty cultures of *V. cholerae* were obtained from this outbreak; they were all of the Ogawa type. This accords with the observations of the years 1936 and 1937, when most of the isolations were made during the period of epidemic prevalence, and all the vibrios isolated from this region were of the Ogawa type. But the vibrios isolated in 1938 during a period of low incidence from sporadic cases and limited outbreaks were of the Inaba type. It was suggested that the change in the prevalent type of vibrio was related to the low incidence of cholera, and that Ogawa would probably re-establish itself in this area during winter if an epidemic should break out. This has been borne out by these observations.

The province has been comparatively free for the rest of the year. Five strains of vibrios isolated in Kurnool district during November were also of the Ogawa type. The significance of this is not quite clear. Both Ogawa and Inaba vibrios have been met with in the Ceded districts in previous years, though at different periods.

Field observations in the Cauvery delta.

During the period the enquiry had been located in Tanjore there was no cholera in the district. The observations were limited to an examination of specimens of water from several sources in two selected areas and of stool specimens of people using these sources. A beginning has been made in the examination of the blood of persons living in this area for the presence of agglutinins against *V. cholerae*. The villages included in this area have been among the first to report cases in previous years and have generally had a severe experience of epidemic cholera.

In the examination of water, large specimens were tested—*viz.* 2,500 ml. quantities—and the mannose-bismuth-sulphite method of enrichment adopted. In control experiments, the technique proved adequate in the isolation of *V. cholerae* when present in small numbers along with numerous non-mannose fermenting NAGs and coliform organisms. One hundred and sixty three specimens have been examined—100 from tanks, 31 from rivers and canals and 32 from wells. The AG vibrio was isolated on only one occasion—from a pond which proved negative on a second examination done two days after. Mannose-fermenting NAGs were recovered from practically every tank and river water examined. The mannose-bismuth-sulphite method of enrichment adopted is not capable of yielding a differential isolation of *V. cholerae* in these circumstances. Attempts to improve this medium by the incorporation of the total polysaccharide fraction of the agglutinable vibrio in the medium in the place of mannose have not proved successful.

Wide variations were noted in the total solids, oxidisable matter and chloride content of the waters in the early part of the investigation, but with the onset of the rains there was a tendency towards greater uniformity. The average figures are: 313 parts per million (total solids), 3.08 p.p.m. (oxidisable matter), and 102 p.p.m. (chlorides) for tanks, 171, 1.45 and 22 for rivers and canals and 646, 0.72 and 232 for wells.

One thousand five hundred and ninety-three stool examinations have been done. An attempt was made to include as large a number as possible of persons who had suffered from cholera during previous outbreaks. In no instance was an agglutinable vibrio isolated. Non-agglutinable vibrios were recovered from 58 specimens. It is to be borne in mind that these observations have been made during a period of complete freedom from cholera of a duration unusually long for this district.

One hundred and eighty-eight specimens of blood from healthy individuals were put up for agglutination against formalised agar washings and steamed agar washings of Inaba and Ogawa. A considerable number of these persons had in previous years received inoculation against cholera. Thirteen of these had been attacked during previous epidemics and 17 were household contacts of these. Agglutination against formalised suspension of Inaba or Ogawa in a dilution of 1-20 was noted in 49

cases. Agglutination to a titre of 80 was noticed in 4 cases. No agglutination was noted with steamed suspensions.

Laboratory experiments were carried out to determine the period of survival of agglutinable vibrios in salt water. It was noted that with a minimal inoculation of *V. cholerae* in water containing 2 per cent common salt they survive for at least 74 days (total period observed). In one per cent salt solution they were recovered up to 54 days and in 3 per cent salt solution they could not be recovered after 32 days. In 2 per cent salt solution inoculated simultaneously with a non-mannose-fermenting vibrio and aerogenes, the agglutinable vibrio could still be recovered after a month.

In preliminary experiments with stool specimens, buffered with a boric-acid potassium chloride buffer at pH 9.0, artificially infected with a minimal inoculum of *V. cholerae*, the vibrio could be isolated up to 12 days.

3. Cholera statistical enquiry under Dr. R. B. Lal at the All-India Institute of Hygiene and Public Health, Calcutta.

Analysis of the Field Data.

The analysis of a large amount of data, which had been accummulated in the course of the cholera carrier & epidemiological enquiry in Diamond Harbour area, was commenced. After completing the transfer of data to punched cards, which occupied a considerable time of the enquiry, an investigation into the general health condition of the population was carried out as this was considered a necessary preliminary to the cholera enquiry proper. The population was divided on a simple basis into four classes *viz* ., (*i*) well, *i.e.*, those who had no complaints at the time and looked healthy, (*ii*) indifferent health, *i.e.*, those who though not actually sick did not feel quite fit and did not look active and bright, (*iii*) actually sick at the time of the visit and (*iv*) suffering from chronic illness.

Of the total population of 10,218, 90% were well, 4% in indifferent health, 1% actually ill and 5% were victims of chronic diseases. Unfortunately it is not possible to compare these figures with those of other populations but the criteria of classification being simple, it is hoped that comparative figures may be available from other sources in future. A comparison of these figures with those obtained from a non-endemic area of Bengal would be of special interest. The analyses were carried out in further detail the results of which are summarised below.

1. *Age and health.* Nearly 95% of the infants were in good health, about 2% were actually ill and a little less than 2 and a half % suffered from chronic illnesses. In the pre-school age, chronic and acute illnesses claimed 6% and 3% respectively. The school age was the healthiest group, chronic illnesses being a little under 2%

and acute illness a little above 1%. Over 95% of the children were in good health. In adolescence the percentage of healthy persons was not very much lower (94.6%). Acute illness was relatively less (1%). With a further advance in age, *i.e.*, beyond 25 years there was a steady decline in the proportion of healthy people and a progressive increase in the chronically sick and the near-normals. In the full working age period, *i.e.*, 25-55, the percentage of chronically ill was as high as 7 and of near-normals 4. Of the people over 65 years, 56% were well, 32% were in indifferent health and 10% were chronically ill.

2. *Health and economic condition.* A rough and ready method of classification was adopted. The basis was the kind of taxes paid by the family. The large majority of people (77%) paid only Union Board tax while 22% paid no taxes but were not indigent. Considering the low economic qualifications required for the payment of Union Board tax, it may be concluded that the economic level of the population as a whole was very low. In the majority group, *i.e.*, Union Board tax payers, nearly 11% were either ill or in indifferent health. Chronic illness accounted for 5% and acute sickness for over one and a half %. It is rather strange that the non-tax payers were a little better off than the above group particularly in respect of chronic illness and near-normal condition. The percentage of the healthy was as high as 92. Other groups are too small to be discussed in detail but it may be mentioned that all the 42 indigents were well.

3. *Health and occupation.* The population was divided into 20 occupational groups. Of these the largest groups were :—housewives (30%), at-home (29%), student-clerk (14%), cultivators (14%), menial servants (5%). The percentages of those who were well, in indifferent health, acutely ill and chronically ill, are shown below in tabular form for each of the major occupational groups. The group 'at-home' is necessarily influenced by peculiarities of age, and that probably accounts for the lowest number of healthy persons and for large numbers of persons in indifferent health or chronically ill. Of the other groups, the majority of whom would presumably be of middle age, the cultivators appear to be at a disadvantage particularly with regard to chronic illness. This is a serious matter and calls for further investigation. It is more than likely that economic factors enter into consideration here.

	Well	Indifferent	Acutely ill	Chronically ill
Housewife	88.0	4.8	1.2	6.0
At-home	89.7	4.0	2.4	3.9
Student-clerk	94.0	2.0	1.3	2.8
Cultivators	90.0	2.8	1.7	5.5
Menial servant	94.1	1.3	1.3	3.2

4. *Health and bowel movement.* At the time of investigation, 51% of the population was found to be in the habit of moving their bowels once a day, 44% twice a day and only about 5% more than twice a day. In the healthy group, the distribution was not materially different from that of the general population but in the group of those who were chronically ill, there was a definite tendency for greater frequency of bowel movements; more than a third (35%) of the

group had two motions a day and more than a quarter (30%) moved their bowels more than twice a day.

5. *Health and appetite.* Ninety-five per cent of the population experienced good appetite and only a very small fraction bad appetite. These latter belonged mostly to the sick groups. Amongst the chronically ill and the near-normals large percentages, 65 and 68 respectively, had indifferent appetite.

6. *Health and gastro-intestinal complaints.* The gastro-intestinal complaints noted were acid eructations, biliousness, biliary colic, jaundice, worms, diarrhoea and dysentery. The percentages of the population subject to these complaints were for acid eructations 2.23, biliousness 0.21, biliary colic 0.05, jaundice 0.33, worms 0.35, diarrhoea 0.58 and dysentery 0.15.

Nearly 1% of those who were in the healthy group had been subject to one or the other of these complaints, while only 0.15% of the acutely ill complained of these conditions, but in the chronically ill group the percentage was nearly as high as 60, and in the 'near-normals' group about 38.

The above discussion makes it evident that in this endemic area there was considerable evidence of gastro-intestinal disturbance, and this fact may have a definite bearing on the cholera history of the community.

Cholera forecast.

In the report of the work done during last year it was mentioned that the method elaborated for forecasting cholera in Calcutta was applied to the homogeneous cholera districts. The method was found suitable in 10 out of 19 districts. The results of this work are presented below in the form of regression equations, where 'y' denotes the expected number of total deaths from cholera in any year and 'x' denotes the cholera mortality for 45-52 weeks of the preceding year.

Homogeneous district No :

2	$y = 175.18 + 2.32x$
4	$y = 16749.74 + 3.06x$
8	$y = 1293.08 + 4.28x$
9	$y = 8847.26 + 9.81x$
11	$y = 336.07 + 8.13x$
13	$y = 1562.99 + 4.18x$
14	$y = 1973.19 + 2.51x$
15	$y = 25.98 + 3.09x$
17	$y = 3101.79 + 6.07x$
19	$y = 26.47 + 3.68x$

4. Cholera vibrio enquiry at the Central Research Institute, Kasauli.

No special staff was provided for this enquiry but work was carried out by the staff of the Institute who had previously been associated with

the enquiry, the total grant received being Rs. 400/- for the cost of media, experimental animals etc.

The work undertaken consisted of :—

(a) detailed examination of Vibrio strains for the Bengal field cholera enquiry.

(b) investigations on certain factors in the growth of vibrios and the production of haemolysin and other points.

Strains isolated from 16 stool sources and 61 water sources were dealt with, multiple separate colonies being examined in many cases. The examinations made consisted of :—

(a) serological tests for H agglutination and for O agglutination with Inaba and Ogawa type sera and in some cases with special O test sera,

(b) sugar reactions for Heiberg type,

(c) tests for production of haemolysin.

The results of examination of strains are tabulated as follows:—

V. cholerae isolated from

Water								Stools							
Sub-group Inaba				Sub-group Ogawa				Sub-group Inaba				Sub-group Ogawa			
Total	Haemolytic	Non-haemolytic	Haemolytic and non-haemolytic	Total	Haemolytic	Non-haemolytic	Haemolytic and non-haemolytic	Total	Haemolytic	non-haemolytic	Haemolytic and non-haemolytic	Total	Haemolytic	Non-haemolytic	Haemolytic and non-haemolytic
3	1	2	—	58	33	20	5	—	—	—	—	16	1	15	—

The majority of the strains examined were of the Ogawa sub-type and the greater proportion of these were haemolytic. In some cases from the same culture both haemolytic and non-haemolytic colonies were obtained by plating.

A technique was devised for the quantitative estimation of haemolysis. For this purpose a solution of acid haematin was prepared by acid haemolysis of goat's red blood corpuscles diluted until it matched the standard of a Sahli haemoglobinometer. The vibrio haemolysis test was carried out with 1 c.c. of 10% goat cells and 1 c.c. of culture. On completion of the test the unhaemolysed red cells were separated by centrifuging and the fluid portion (from 2 c.c.) diluted to 8 c.c. with water and normal HCl. This was compared with a threefold dilution of the standard in a colorimeter. Employing this technique, strains were shown to vary in haemolysin production from time to time, some haemolytic strains becoming completely non-haemolytic. Colonies from the same culture

varied in their degree of haemolytic action. It is understood that the strains examined of the Ogawa sub-type, both haemolytic and non-haemolytic, were mostly isolated from tanks in the absence of cholera cases and the findings will require to be interpreted by the field enquiry in relation to their work.

Many points were observed in the course of the examination of haemolytic agglutinable strains which indicate the necessity for further investigation of the material collected and strains are being maintained for the purpose.

The production of haemolysin in 1% peptone water by a number of strains including Egyptian El Tors, Celebes El Tors and inagglutinable haemolytic strains was studied under different conditions of initial pH with and without a buffer. The range of pH was 6.2 to 8.4. Most active haemolysin production occurred after 3 days with initial pH of 7.0 and during the period of incubation pH rose to 8.0. The presence of a buffer made no difference in haemolysin production. Haemolysin production was equal in Douglas Broth.

Gispen (Ann. Inst. Pasteur, 63, 3rd Sept. 1939) has claimed that El Tor strains could be differentiated from typical *V. cholerae* by the fact that after heating at 56° to 57°C for 3 hours the El Tor suspensions remained agglutinable while *V. cholerae* suspensions lost their agglutinability. This was tested in the case of a series of 11 strains and not confirmed.

5. Cholera bacteriological enquiry under Major C. L. Pasricha, I.M.S., at School of Tropical Medicine, Calcutta.

Vibrios from Cholera patients.

During the first half of this year 173 cholera patients were examined. The isolations were made after enrichment in (*a*) peptone water and (*b*) modified Wilson and Blair's medium followed by plating on Aronson's medium and also on bile-salt agar. Enhancement in Wilson and Blair's medium gave better results than enhancement in peptone water. Agglutinable vibrios were isolated from 74 or 43 per cent of the cases. Hagg vibrios (H agglutinable) and Nagg (not agglutinable with Inaba H & O serum) were isolated from 4 per cent and 12 per cent of cases respectively.

The serological types of vibrios isolated from clinically cholera patients during the year ending 28th February 1939. During this period the stools from 959 clinical cases of cholera were examined. Vibrios were isolated from 438 or 45.7 per cent of the patients examined. The Inaba type of *V. cholerae* was isolated from 60.7 per cent of the bacteriologically positive cholera cases. The Ogawa type of *V. cholerae* was present throughout the year. There was no appreciable preponderance of the Ogawa type at any particular time of the year or at the cholera epidemic. The H agglutinable vibrios, *i.e.*, vibrios inagglutinable with Inaba O or Ogawa O sera but agglutinable with H & O serum were found in 2.8 per cent of

the patients from whom vibrios were recovered. The inagglutinable vibrios were found in 10.7 per cent of the cases. (Indian Med. Gaz. Vol. LXXIV, 1939, p. 680).

Although there are 13 types of choleraphage a search for the different types in the stools of six cholera patients failed to reveal any type except type A. This type is the most widespread type and can be isolated from natural contaminated waters, flies and from a certain number of cholera strains.

CHOLERA AGGLUTININS IN PATIENTS AND HEALTHY PERSONS.

Sera were examined from 26 cholera convalescents and 96 healthy individuals who had not had cholera, or been in contact with cholera cases and had not been inoculated with cholera vaccine. The antigens used were (1) Kasauli 1800/1—Inaba type—"Vaccine strain", (2) Kasauli 38,107 —Ogawa type "Vaccine strain", (3) Calcutta Inaba type 1993 (isolated in 1937). The suspensions were made (1) 0.2% formolized saline suspension of 18 hours' agar growth and (2) boiled saline suspension of 18 hours' agar growth.

The outstanding points were :—

1. agglutinins for the vaccine strains (Kasauli 1800/1 and 38,107) were present in low titre and were absent in a number of cholera patients.
2. V. 1993 was the best strain for the detection of agglutinins in cholera patients but not in healthy individuals.
3. better results were obtained by using Felix tubes than with Dreyer's tubes.

In 71 healthy individuals (both the Dreyer's method and Felix tubes were used) the following results were obtained.

(*i*) *Dreyer's method.* Only one of the 71 individuals examined showed cholera agglutinins in dilution of 1 in 25.

(*ii*) *Felix's method.* 71 healthy individuals examined.

Dilutions	Strain used (living culture.)		
	Kasauli Inaba 1800/1	Kasauli Ogawa 38107	Calcutta Inaba type 1993
Positive in 1 in 25	60 or 84%	17 or 24%	45 or 63%
Positive in 1 in 100	18 or 25%	2 or 3%	7 or 10%

Interesting results have been obtained by watching the agglutination reactions of freshly-isolated strains of *V. cholerae.* With the Inaba and Ogawa sera, agglutinable vibrios (using formolized suspension) fell into the following groups.

1. Inaba +, Ogawa —
2. Inaba + or ±, Ogawa +
3. Inaba —, Ogawa +.

The Ogawa serum used was completely differential but not the Inaba serum. The majority of the laboratory strains of *V. cholerae* fell into the first two groups. A few of the freshly isolated strains fell into the third group *i.e.* Inaba —, Ogawa +. Two such strains which were very carefully examined were found to change their agglutination reactions and to fall into group 2 *i.e.* to become Inaba ±, Ogawa +. These strains were studied carefully by three independent workers with identical results. Another point of importance brought out in this work is that, whereas Ogawa serum is differential when formolized suspensions are used, the same serum is not differential with boiled suspensions. Further, a boiled suspension of a Nag case strain (No. 1140) was agglutinated by Ogawa serum when a boiled suspension was used for the test and this strain removed the agglutinins (? non-specific) for Inaba type from the Ogawa serum leaving the Ogawa agglutinins unaffected. This strain (No. 1140) is inagglutinable when formolized suspensions are used.

Vibrios from natural waters.

Agglutinable vibrios were isolated from four of the 77 samples of natural waters examined. The samples were collected from areas where there were cases of cholera in the neighbourhood. There was evidence of recent contamination of the source of supply with cholera material. The isolation was made on modified Wilson and Blair's medium. Parallel inoculations on other media were negative. Nagg or Hagg vibrios were present in many of the samples.

The viability of *V. cholerae* in samples of waters that had not been treated in any way was found to vary from 1 hour in samples of hill water to 72 hours in water collected from certain tanks. These tank waters were found to contain a considerable amount of organic matter and to have a high salt content. The viability of *V. cholerae* is considerably increased in autoclaved samples. A paper on this subject was published in Indian Med. Gaz. December 1939.

Testing for potency etc. of cholera vaccines.

As a result of the circular No. 1 of 1939 from the Central Advisory Board of Health pointing out the necessity for using only genuine vaccine, samples of cholera vaccine submitted by different sources have been tested for sterility and potency. The suitability of certain freshly isolated strains for vaccine preparation is being examined.

The ordinary bazar type of fowl (costing in Calcutta approximately 7 annas each) was used for raising serum. The injections were given in the wing vein. Satisfactory serum (titre 1 in 2,500) was obtained using Inaba O, Ogawa O antigens. There is not only considerable economy in the use of the domestic fowl but the availability of these animals and the ease with which they can be fed and cared for make them particularly useful. A medium-sized fowl weighing 1¼ to 1½ lbs. can be bled 10-15 c.c. (conveniently with a syringe) from the wing vein. The domestic fowl is particularly valuable in testing cholera vaccine for which a number of animals is required. (Indian Med. Gaz. December 1939).

Chemical studies.

Seventeen cholera patients were studied. Blood was collected in the acute stage of the disease soon after admission to the cholera ward

and before any intravenous injections had been given. The following examinations were made, cell volume (centrifuge method), haemoglobin (Newcomer's method) moisture (Bang's method), glucose (Hagedorn and Jensen's method), urea (modified Xanthydrol method), sodium chloride (modified open Carius method), non-protein nitrogen (micro method), fibrin and globulin nitrogen (micro method), and inorganic phosphate (Youngberg & Youngberg's method). The results obtained were :—

	Limits	Average	Nominal Limits	Average
1. cell volume	36. 3-69.3	50.5 per cent	36. 0-49.8	43.1
2. haemoglobin	8.25-26.2	17.75 gms per 100 cc. of blood	13. 4-18.1	15.3
3. moisture	73. 3-81.6	77.45 „ „	75 - 82	78
4. glucose	90 - 243	152 mg. „ „	80 - 120	100
5. sodium chloride	298 - 496	435 mg. „ „	450 - 500	475
6. urea	28 - 125	62 mg. „ „	19 - 39	26
The following constituents of plasma were estimated.				
1. moisture	85. 5-92.7	89.9 gms per 100cc. of plasma.	90 - 91.5	90.5
2. glucose	76 - 286	152 mg. „ „	80 - 120	100
3. sodium chloride	491 - 640	586 mg. „ „	518 - 670	600
4. protein nitrogen	1078 - 2139	1538 mg. „ „	928 - 1376	1120
5. non-protein nitrogen	28 - 145	60 mg. „ „	18 - 38	30
6. fibrin nitrogen	51 - 153	94 mg. „ „	32 - 64	—
7. globulin nitrogen	495 - 1348	812 mg. „ „	192 - 464	400
8. albumin nitrogen	243 - 1147	652 mg. „ „	608 - 1040	740
9. inorganic phosphate	3.9 - 17.3	7.2 mg. „ „	2 - 5	4

There is an increase in blood urea, sugar, inorganic phosphate, total proteins, globulins and a relative decrease in chlorides (less marked in plasma chlorides). These findings are similar to those that occur in secondary shock. This conception as the underlying cause of the symptoms during the acute state of the disease would explain the remarkable result in the condition of the patient after an intravenous saline.

The fibrin nitrogen, albumin nitrogen, globulin nitrogen are increased. The increase in globulin nitrogen is more marked than the increase in albumin nitrogen with the result that the albumin-globulin ratio is disturbed.

VI ANTIGEN.

As a preliminary to the study of this antigen in cholera it was thought advisable to work with Vi strains of Bact. typhosum. The strain "Vi I (Bhatnagar)" and "Vi phage" of Craigie and Brandon was kindly supplied by Major Bhatnagar from the Central Research Institute. It was found that type I typhoidphage (described in 1937) corresponded to Craigie and Brandon's Vi phage. The four types of phages isolated in Calcutta act well on "Vi I strain" and the resistant culture which develops after the action of type I phage is no longer agglutinable with Vi serum. The effect of other phages on this strain is being studied. The Vi I strain now shows well marked motility (in semi-solid agar and under the microscope).

6. Cholera toxin and immunity enquiry under Dr. H. Ghosh, Indian Institute for Medical Research, Calcutta.

ENDOTOXIN.

The susceptibility of skin positive rabbits to the endotoxin of *V. cholerae* as noted previously in last year's report was further studied.

Polysaccharides of agglutinating and non-agglutinating types of *V. cholerae* showed identical skin reactions in rabbits and those giving positive skin reactions succumbed in the same way when endotoxin was given intravenously.

Reaction to intracutaneous inoculation of the endotoxin of *B. coli* was also observed. It produced the same kind of reaction as with the endotoxin and polysaccharide of *V. cholerae* and the rabbits showed similar susceptibility to intravenous injection of the endotoxin of *V. cholerae.*

The same susceptibility of rabbits resulting in death was also observed when 4 days broth culture filtrate of *B. coli* was injected intravenously to the rabbits showing skin positive reaction to the endotoxins either of *V. cholerae* or *B. coli.*

The skin test with histamin-hydrochloride was also tried, but it did not show any special skin sensitiveness in any particular groups of animals as was observed with the endotoxin and polysaccharide of *V. cholerae.* It seems, therefore, that the skin sensitiveness might be due to the susceptibility of some rabbits to the polysaccharide present in *V. cholerae* or *B. coli.*

Endotoxin of both agglutinating and non-agglutinating types of *V. cholerae* behaved in the same way so far as the skin sensitiveness and susceptibility of rabbits are concerned.

All the known methods of isolating and extracting the endotoxin of *V. cholerae* were adopted and tried for toxicity. The result was the same except that the dose varied.

NATURE OF GROWTH OF *V. cholerae* IN BROTH HAVING DIFFERENT pH AND WITH OR WITHOUT SUGARS, WITH REFERENCE TO TOXIN PRODUCTION.

In ordinary broth without sugar having pH 8.9 to 9.5, the growth was slight. When pH was kept between 7.1 and 8.9, the growth varied from fair to good, the best growth having been obtained with pH of broth at about 7.8. Below pH 7.1 growth was fair but below 5.2 there was no growth.

In all cases the pH of the culture dropped after 24 hours' incubation. The degree of fall of pH depended on the initial pH and the intensity of growth.

In broth with sugars, *V. cholerae* grew abundantly. It was noticed that when 0.2% glucose or maltose was added to ordinary broth hvaing pH between 7.4 and 9.2, the growth used to be abundant within 18 to 20 hours, the pH dropping down to 6 or below. The growth settled down, the supernatent broth being clear after 24 hours' incubation. On further incubation the pH remained stationary and after 5 or 6 days' incubation no growth of *V. cholerae* was obtained on sub-culture.

The filtrate of the culture was almost non-toxic. It was, therefore, evident that the acid reaction of the media formed during growth, destroyed both the vibrio and its toxigenic property so far as agglutinating variety was concerned.

One outstanding difference was noticed here between the character of agglutinating and non-agglutinating types of vibrios. When the pH dropped down to 6 or below (culture in broth with sugar) the non-agglutinating types of vibrios survived in culture and showed some further growth, the pH of the culture media having been found to go upwards gradually, the agglutinating type invariably died and there was no change of pH of the media. This phenomenon clearly indicates the greater power of resistance of non-agglutinating types of vibrio than that of the agglutinating types.

In order to alter growth without letting the pH to drop to acid side and in order to study the toxicity of such broth culture filtrate the following means were adopted.

1. *Addition of buffer salts such as disodium hydrogen phosphate and sodium acetate in* 0.2% *glucose broth.* The fall of pH of the culture to the acid side could not be prevented by the addition of any of the above buffer salts.

2. *Culture in glucose broth in cellophane or parchment paper bag dipped in a receptacle containing the same broth having higher alkalinity.* The experiment failed repeatedly due to the passage of the vibrio through the bag contaminating the outer broth.

3. *Addition of* 2% *calcium carbonate to the broth having pH* 8.6 *and* 0.1% *glucose,* 0.3% *maltose and the buffer salts.* In this medium the reaction of the culture could be prevented from dropping below pH 7.2 even after 24 hours' incubation. Besides exuberant growth could be obtained. As incubation proceeded, the pH mounted up to 9.3 within 8 or 9 days. The growth used to be diffuse, scum began to form after 24 hours which became thicker and thicker as incubation proceeded.

4. *Modification of the above medium with pH at* 8 *and without sugar and calcium carbonate but with the buffer salts.* The growth in this medium was the same as above the only difference being that the growth became profuse gradually.

Tests of toxicity on animals of filtrates of different broth cultures as mentioned above.

The broth culture after different days of incubation were filtered through L_3 candle and then used for toxicity tests.

In broth prepared according to the method No. 4, the toxin production seemed to be the highest when agglutinating Inaba strain was used. The best toxicity was obtained when the pH of the culture medium mounted up to 9.1 or 9.3, which took place after 7 days' incubation.

If at any time during the period of incubation the pH happened to drop down below 7.2 the toxin production was nil. For the production of the best toxin the growth must be diffuse and a scum formation was essential.

Toxicity test of broth filtrate on guinea-pigs by intravenous method. Guinea-pigs of weight between 180 and 220 gms. were used for all experiments.

When the growth, scum formation and pH were satisfactory, the minimum lethal dose of the filtrate of 7 days' culture in broth, prepared according to the method No. 4, was found to be 0.25 c.c. uniformly, death taking place within 24 hours. The experiment was repeated several times using 6 guinea-pigs for each lot of toxins and the results were corroborative

With toxin prepared in the same way with non-agglutinating (2 strains of non-agglutinating types of vibrio) vibrios the minimum lethal dose for guinea-pigs was never less than 1 c.c., thus showing a distinct difference in toxin formation of the 2 types of vibrio. Further experiments with a large number of non-agglutinating types are being continued.

Symptoms and post-mortem findings in guinea-pigs after injection of broth filtrate. After intravenous injection the guinea-pigs became sick within 1 to 2 hours. They lay prone and flaccid on the floor of the cage, the head dropped forwards. The abdomen became distended, doughy and apparently tender to touch. The respiration became deep and before death it consisted of short, sharp jerks. In some cases twitching movements and spasm of the body were noticed before death.

On post-mortem examination, in almost all cases congestion and distension and in a few cases even rupture of the stomach were noticed. There was excess of fluids in the stomach and in the peritoneal cavity, the latter being sometimes tinged with blood. In cases where death took place within 24 hours, the small intestine was found to be congested and to contain liquid stool. The supra-renal glands were generally congested.

Toxicity test of broth filtrate on rabbits by intravenous method. Some of the rabbits showed extreme susceptibility and died within 1 or 2 hours, while others survived even when higher doses were injected. Consequently it seems that the rabbit is not a suitable laboratory animal for experiment with toxin of *V. cholerae.*

Studies on the character of the toxins.

The studies so far made indicated that, when the minimum lethal dose of the toxin (filtrate of broth culture of agglutinating Inaba strain) was 0.25 c.c. in guinea-pigs on the same day of filtration, there was distinct deterioration of the toxin by keeping it for 3 days. After 3 days the minimum lethal dose of the same toxin was found to be 0.5 c.c. instead of 0.25 c.c. The action of heat on and other characters of the toxins are being studied.

Attempts are now being made to obtain a more potent toxin and to study the difference between agglutinating and non-agglutinating types so far as toxin production in liquid media is concerned.

7. Cholera kidney enquiry under Dr. D. N. Bannerjee at the Carmichael Medical College, Calcutta.

The clinical picture of fatal cases of cholera from renal failure invariably shows profound toxic manifestations. Curiously, the pathological changes in the kidney are very slight and inconspicuous when examined by ordinary methods. The co-relation of the clinical manifestations of renal failure in cholera and the histological changes of the kidney is found to be extremely obscure.

The great retention of NPN, urea, uric acid, creatinin etc. in the blood in cholera cases is a very constant and prominent finding. It has further been emphasised that the loss of chloride and loss of fluid from the body play a great role in the production of the symptom complex of renal failure in cholera.

With this point of view the problem of cholera kidney has been studied under two heads :—

1. the renal factor.
2. extra-renal factor.

The renal factor in cholera kidney.

As our post-mortem examinations are generally done at least 24 hours after the death of the patient, and as cholera post-mortems are done usually in the summer months, well marked post-mortem changes are invariably present which make specimens often useless for further study. In order to obviate this defect, kidneys from 26 cholera cases were removed within half an hour after death and the material immediately fixed in different fixatives.

In addition to these, specimens of kidney were obtained from cases of bacillary dysentery for comparison with cholera. These specimens were examined by various histological methods.

Capillary activity in cholera kidney. The examination of these specimens shows wide-spread dilatation of the capillaries both in the glomeruli and in the interstitial tissues. Numerous areas are seen in which red blood cells have come out of the vessels indicating gross damage to the endothelial lining. Degenerative changes in the tubules and, by special staining of the basement membrane, some glomerular changes are observed.

The great depletion of the interstitial fluid in cholera is undoubtedly due to the capillary walls becoming unduly permeable, particularly of the alimentary tract, causing intense vomiting and purging. In the kidney, as in other organs, the capillary activity is well pronounced. The nature and extent of the capillary permeability in cholera kidney has been estimated by the protein content of the urine. When the capillary permeability is slightly increased a trace of protein is found in urine ; when it is greater, the protein content may become 0.5 grm. per cent while in extreme cases the wall becomes so permeable as to show presence of red blood cells in the urine. In cases of extreme damage to the wall haemorrhage is found to take place when examined histologically and that indicates the presence of necrosis of the capillary wall. The glomeruli show great

engorgement and presence of red blood cells inside the Bowman's capsule which is further corroborated by the presence of albumin and R.B.C. in urine.

Normally, all the glomeruli are not functionating at the same time. The estimation of active and inactive glomeruli varies widely. The capacity of the whole capillary network in the kidney at rest and in cholera varies greatly. This depends on the intrinsic tone of the capillaries in regulating the flow of blood. Like the arterioles, the capillaries also possess a motor nerve supply from the sympathetic system, and the contractility of the capillaries depends on the presence of the cells of Rouget and Meyer which are flat, branching, contractile cells situated externally to the endothelial wall and encircling the capillaries with fine processes. The presence of the continuation of this contractile coat with the plain muscle coat of the arterioles on the one hand and the venules on the other is responsible for the fine adjustment of the renal circulation.

For this reason, in the present study, the arterioles, capillaries, and venules have been included as one unit responsible for the vascular activity in cholera kidney. Comparing with normal kidney, it is found that the capillary bed which had remained inactive in normal condition came into activity and also widely dilated. This has also been observed in other organs in cholera and that has been widespread and conspicuous.

One can assume from this fact that a considerable portion of the total blood, still remaining after the profound loss of the interstitial fluid, is used to fill up the dilated capillaries and so contribute to a great extent to the production of low blood pressure and incidentally to a vicious circle in the renal function.

The capillaries of the kidneys in cholera are not only dilated but have also lost their tone and have become abnormally permeable, particularly those of the glomeruli.

The fall of general blood pressure is essentially not due to failure of the cardiac musculature but to a vasomotor collapse due to oligaemia aggravated by the huge loss of fluid through the increased permeability of the intestinal capillaries. The renal vascular failure in cholera is only a part of the whole systemic peripheral capillary failure. The arterial system has been found to be empty while the venous system, particularly of the splanchnic area engorged. This leaves the capillary system stagnant and the blood pressure remaining as low as before. This explains those cases in which the effect of intravenous injection is often transitory as if the intravenous fluid is poured into a hollow organ. Permeability through the endothelial lining plays a great role in the change of the interstitial fluid in cholera. Attempts are now being made to investigate the methods for the study of this problem.

The structure of cholera kidney when still functionating. In order to study the structure of the cholera kidney when it is still functionating, advantage is taken of the fact that in the frog, the glomerulus gets its blood supply from the renal artery, while the tubule gets its supply from the renal portal system. Perfusion experiments were done in the living frog with the addition of vital dyes.

The experiments were arranged in the following different sets :—

1. Neutral red is allowed to pass through the glomerulus.

2. Neutral red through the tubules.
3. Janus green through the glomerulus.
4. Janus green through the tubules.
5. Neutral red through the glomerulus combined with Janus green through the tubules.
6. Janus green through glomerulus combined with neutral red through the tubules.

These different combinations of the two vital dyes stained the different functionating cells of the two portions of the nephron and have given very interesting findings. These are now being compared with similar sets of experiments on frogs which received sublethal and lethal doses of the comma vibrio. This study of morphological and functional changes of the cholera kidney is in progress and very promising results are being expected.

By this course of study and also by intravitum injection of trypan blue in normal and inoculated mice, it appears that, by elaborating suitable methods, the process of the cellular mechanism of renal function in cholera can be appropriately studied.

Extra-renal factors in cholera kidney.

From a study of the problem of the cholera kidney during the last few years it has been found that the renal failure is greatly due to some extra-renal factor, of which the loss of chloride and haemo-concentration play the important role. In order to study this part of the problem 20 cases of cholera, diagnosed clinically and bacteriologically, have been examined thoroughly. The blood, stools, vomitus and urine of these cases have been examined every day from the day of admission to convalescence or death. The total quantity of the stools, vomitus and urine have been collected in separate jars for 24 hours and preserved with thymol. The total quantity was measured and a sample taken for examination for chlorides, protein, NPN, urea, uric acid, etc. From these 20 cases 68 samples of blood have been examined for estimation of chlorides, NPN, uric acid, and serum protein; 58 samples for estimation of urea; 66 samples of stool have been examined for chlorides, NPN, uric acid and protein; 52 samples of stools for estimation of urea; 43 samples of urine examined for estimation of chlorides and uric acid and several samples of vomitus have been examined for chlorides.

For the study of haemoconcentration in cholera, these 20 cases have been examined daily for (1) corpuscular volume by haematocrit and (2) estimation of haemoglobin.

Haemoconcentration in cholera in the production of renal failure. Loss of fluid is undoubtedly a very important factor in the production of renal failure in cholera but cases are not uncommon in which replacement of the lost fluid does not relieve anuria and prevent death from renal failure.

The fluids of the body consist of about 70% of the body weight and are contained in three compartments in the body, *e.g.*, blood plasma 5%, the interstitial fluid 15% and the intracellular fluid 50% of the body weight. The interstitial fluid lies between the vascular compartments and the tissue cells and includes the lymph.

In cholera, as it is extremely difficult to take the body weight during the active stage of the disease, the body weight has been taken to be about 55 kilo for an adult. Calculating on this basis, the blood plasma constitutes 2.75 litres, the interstitial fluid 8.25 litres and the intracellular fluid 27.5 litres. The normal blood volume of an adult generally consists of 5% to 8% of the total body weight, *i.e.*, 2.75 to 4.4 litres, averaging 3.5 litres. It has, further, been observed that fairly severe dehydration indicates a fluid loss of at least 6% of the body weight, *i.e.*, for an adult weighing 55 kilo, the fluid loss amounts to about 3.3 litres.

In cholera the dehydration is severe, so a loss of about 3.3 litres can be taken to be a common occurrence. Comparing these two figures it appears that the fluid loss in cholera amounts to the total volume of blood present in the individual.

This drainage is mainly from the interstitial fluid which is the intermediary between the blood and the intracellular fluid helping the exchange of fluid between the two other components. In cholera the great loss of interstitial fluid amounting to 6 to 8 per cent., *i.e.*, nearly the half of the total quantity of the interstitial fluid, cannot compensate the depleted plasma volume, as shown by the invariable decrease of corpuscular and plasma volume. This must also influence by hindering the fluid exchange to the tissue cells leading to serious consequences.

An attempt has been made in the present study to know (1) quantity of fluid the body loses in cholera of average severity, (2) the amount of water required by the patient for the restoration of body fluids to the normal level and (3) the amount of water required for the normal excretion of the waste materials by the kidneys in cholera.

The daily examination of corpuscular volume by haematocrit and estimation of haemoglobin and specific gravity of blood show that the patients very often present a blood concentration amounting to more than 140% of the normal volume, which figure is set as a dangerous level indicating that a man cannot long survive this amount of blood concentration. Even when the concentration reaches 125% of normal volume, conditions for the maintenance of life become precarious.

It has, further, been found that there is a considerable fluctuation of the haemoglobin figures which is indicative of the unstable equilibrium of the water balance mechanism due to the transference of the interstitial fluid first to restore the intracellular fluid and then to the blood volume. It has been found that an amount of 3.5 litres (6 pints) of water is required by a cholera patient of average severity for the restoration of the lost body fluids to the normal level.

The fluid administered in the form of intravenous saline is mainly utilised to restore to normal some of the internal chemical disturbances. The fluid administered is first used for replenishing the interstitial fluid and only when the interstitial fluid is restored does the surplus fluid administered become available for renal function, in order that a sufficient quantity of urine may be produced for dissolving the waste products of metabolism and for keeping the blood chemistry constant in composition and volume. The urine volume, for this reason, is not found to be increased immediately.

The role of haemoconcentration in anhydraemia, reduction of blood volume and influence on the interstitial fluid and the intracellular fluid and also the role of loss of the interstitial fluid causing disturbance of the acid base equilibrium are under study.

Hypochloraemia. The blood plasma and the interstitial fluid require for their maintenance the three important materials *e.g.* water, Na and Cl. The other materials are supplied by the process of metabolism. In order to study this part of the problem 20 cases of cholera have been studied in the following way :—

The blood, stools, vomitus, and urine of all these cases have been examined for chlorides every day from the day of admission to convalescence or death. The total intake of chloride, in the form of intravenous or subcutaneous injection of saline, has also been estimated.

The sodium chloride content of the blood is found to be invariably low, the lowest figure obtained being 340 mgm. per 100 c.c. of the blood. When compared with the haemoglobin values, the NaCl values showed an inverse relation to those of haemoglobin *i.e.* when the blood was highly concentrated the NaCl content was distinctly low and when the haemoglobin value was not high the NaCl values came near about normal figures. Considerable quantity of NaCl is found to be present in the stools and vomitus.

The problem of the loss of chloride in cholera and its relation to the function of the kidney is still under study.

Retention of waste products. That there is a great retention of urea, NPN etc. in almost every case of cholera has long been observed. It has been observed, further, that this retention is essentially not due to a defect in the function of the kidney in eliminating them but that it is for the purpose of compensating for the altered osmotic pressure resulting from loss of electrolytes. Loss of chlorides and dehydration in cholera are mainly responsible for the concentration of N waste products. The factors governing the rate of excretion of urea, NPN, chlorides etc. in cholera are now under study.

8. The Malaria Institute of India, Kasauli.

STAFF.

(*i*) Lt.-Col. G. Covell, I.M.S., Director, on leave ex-India 5th August to 28th September. (*ii*) Major M. K. Afridi, I.M.S., Assistant Director. (*iii*) Major Jaswant Singh, I.M.S., Research Officer. (*iv*) Dr. I. M. Puri, Entomologist. (*v*) Rai Sahib Dr. Khazan Chand, P.C.M.S. (retd.), Assistant to Director. Major Afridi officiated as Director and Major Jaswant Singh as Assistant Director during Lt.-Col. Covell's period of leave.

TOURS.

(*i*) Lt.-Col. Covell toured in the U. P. from 6th to 9th February in connection with the U. P. rural antimalaria scheme and inspected the work of the field research unit at Bazpur. He subsequently toured in S. India from 18th to 25th April, and visited Coorg and the Wynaad, inspecting the work of the field research unit in the latter area. From there he went on to Ceylon and saw some of the antimalaria work carried out by the Ceylon Government and by the Ross Institute. He then went on to Singapore to lecture at the International Course in Malariology for medical officers and engineers organized by the League of Nations, after which he visited a number of localities in Malaya where antimalaria work is in progress. On his return journey he visited Rangoon and discussed malaria problems in Burma with the Director of Public Health. After reaching Calcutta he inspected the work of the field research unit working on the shore of the Chilka Lake, Orissa, arriving back at Kasauli on 13th June. (*ii*) Major Afridi accompanied the Director on his tour in the U. P. in February, and again toured that province from 19th to 24th April, to visit the field research unit stationed at Bazpur and advise on the work of the U. P. rural malaria scheme. (*iii*) Major Jaswant Singh visited Lahore and Amritsar from 24th to 27th April to collect human blood for preparing anti-sera for the precipitin test of mosquito blood meals. (*iv*) Dr. Puri visited Karachi from 24th April to 9th May to carry out tests on the efficacy of various insecticides in spraying aircraft.

INSTRUCTION.

(*i*) The annual malaria course was held at Delhi from 6th March to 15th April, and was attended by 24 students, of whom 21 passed the examination. (*ii*) Two sanitary inspectors and 1 malaria inspector of the B. B. & C. I. Railway and 1 malaria inspector of the B.-N Railway were given training in practical methods of mosquito control. (*iii*) A study tour in the Wynaad from 4th to 10th June was arranged for Dr. Nair of the Travancore State Health Department. (*iv*) Mr. Menon of the Travancore State Health Department underwent a course of training in medical entomology under the supervision of Dr. Puri from 15th April to 31st July. (*v*) Certain personnel were trained by the 3 field units for the Madras, U. P. and Orissa Governments respectively. (*vi*) A number of unpaid apprentices were trained as insect collectors at Delhi.

PUBLICATIONS, REPORTS, ETC.

(*a*) *Journal of the Malaria Institute of India.* Four numbers containing 35 papers and 1 abstract, have been published during the year.

Of the 36 papers above referred to, the following were by the members of the Institute staff :—

(1) Antimalaria operations in Delhi. Part I. (Covell).
(2) *Aedes* survey of Hog Island. (Afridi).
(3) Treatment of simian malaria with M & B 693. (Jaswant Singh and Harwant Singh).
(4) Food preferences of *anopheles* mosquitoes in the Delhi urban area. (Afridi, Jaswant Singh and Harwant Singh).
(5) Antimalaria operations in Delhi. Part II. (Covell & Afridi).
(6) Malaria in the Wynaad, S. India. (Covell & Harbhagwan).

Health Bulletins.

Published.

(1) H. B. 18. A practical entomological course for students of malariology. (Barraud, revised by Puri).

In Press.

(2) H. B. 14. How to do a malaria survey. (Christophers, Sinton and Covell, revised by Covell).
(3) H. B. 13. Instructions for collecting and forwarding mosquitoes. (Sinton, revised by Puri).
(4) H. B. 5. Lectures on malaria. (Covell).

Other publications.

(1) Note on antimalaria operations in Delhi (Covell). *Jl. of the Institute of Civil Engineers.*
(2) The principles and economics of antimalarial sanitation (Covell). *Jl. Malaya Br. Brit. Med. Ass.*, III, 2.

MISCELLANEOUS REPORTS AND NOTES.

These have been submitted on (1) control measures in Delhi, (2) efficacy of insecticides in spraying aircraft, (3) effect of *Nata Karanja* in treatment of malaria, (4) antimalaria measures in Medoowela, U. P., (5) antimalaria measures in Savantvadi State, (6) antimalaria measures in Baroda, (7) antimalaria measures in Puri, (8) antimalaria measures in Bhavnagar State, (9) antimalaria organization in Burma, (10) synthetic antimalaria drugs produced in countries other than Germany, (11) traps for collecting mosquitoes, (12) larvicidal properties of *Picrasma nepalensis*, (13) value of insecticidal spraying in villages, and (14) use of oil booms for larval drift.

SCIENTIFIC MATERIAL SENT TO OTHER WORKERS.

Monkey malaria strains have been sent to various workers in India and England. Stained blood films showing human and monkey malaria parasites, slides showing oocysts and sporozoites, and *Gambusia* fish have been sent to a number of workers in India.

ROUTINE ENTOMOLOGICAL WORK.

Expert identification of mosquitoes and sandflies has been carried out as in former years, numerous collections having been received from workers in all parts of India. The collection and preparation of large

numbers of mosquitoes for instructional purposes, demonstration, study and exchange have been continued.

Testing of larvicides.

A number of these have been tested under field conditions.

Testing of insecticides.

A number of proprietary insecticides have been tested at Karachi, Kasauli and Delhi. Those containing pyrethrum continue to prove the most effective. Encouraging results have been obtained with samples of pyrethrum flowers grown in Kashmir and Coonoor.

Antimalaria operations in Delhi.

These have again been supervised by officers of this Institute, the methods employed being similar to those practised in previous years. The control of mosquito breeding has been very complete, but it is recognized that the measures taken have been greatly aided by the deficiency of monsoon rainfall.

The library.

This has been extended by purchase and exchange, and various publications have been sent on loan to workers in other parts of India.

The museum.

Numerous photographs illustrating malaria control measures in India and elsewhere have been added.

The field station.

This is now located in Delhi, the move from Karnal having been completed early in the year. Difficulties in administration have been greatly diminished by the move.

Field researches.

(*i*) *Delhi Field Station.* Observations on the bionomics of *A. culicifacies* have been continued. Two villages in the urban area were sprayed throughout the malaria season to test the effect of this measure. Monthly spleen and blood examinations have been carried out in the coolie camps. The efficacy of oil-booms in dealing with larval drift has been tested. (*ii*) *Wynaad Field Unit.* Experiments have been carried out to test the value of herbage packing for controlling the breeding of *A. fluviatilis.* Dissections of this species showed an infectivity rate of 26 per cent during the height of the transmission season. Precipitin tests showed that 97 per cent of this species caught had fed on human blood. (*iii*) *U. P. Terai Field Unit.* Observations on epidemiology have been continued. *A. minimus* has been incriminated as the chief malaria carrier in this area, with an infectivity rate of 18 per cent during the month of October. The effects of automatic sluicing and intermittent irrigation on the breeding of mosquitoes have been studied. (*iv*) *Orissa Field Unit.* Observations as to the amount and seasonal distribution of malaria, identity of the carrier species of anopheline and the bionomics of the local anophelines have been carried out.

LABORATORY RESEARCHES.

(*i*) Observations on the behaviour of monkey malaria parasites have been continued, with special reference to the numerical increase and decrease of parasites. (*ii*) A number of different strains of monkey malaria have been maintained. (*iii*) Anti-cow serum for use in the precipitin test has been successfully prepared. Considerable advances have been made in the preparations of other types of anti-sera for this purpose. (*iv*) 7,000 specimens of mosquito blood meals have been tested by the above method. (*v*) Observations on the agglutination reactions of various anti-sera and antigens in *P. knowlsi* infections have been carried out.

ENTOMOLOGICAL RESEARCH.

Observations have been continued on the bionomics of *A. culicifacies*, *A. stephensi* and *A. fluviatilis*. Several generations of the two latter species have been maintained in the laboratory. The behaviour of mosquitoes under different conditions of temperature, humidity and light has been studied. Extensive observations have been made on the ovarian cycle and the development of the ovarian follicle in mosquitoes, and on the possible function of the oesophageal diverticula.

9. Malaria transmission enquiry under Dr. B. M. Das Gupta at the School of Tropical Medicine, Calcutta.

The work on the physical factors of atmospheric temperature and relative humidity governing malaria transmission by *A. annularis* has been completed. The following report on the species is by Dr. B. C. Basu, D. Sc.

A total of 1,322 laboratory bred clean female *A. annularis* (*fuliginosus*) were fed on 5 gametocyte-carriers (B.T. 2 ; M.T. 2 ; and Q. 1), and immediately after feeding were exposed to the following condition of atmospheric temperature and relative humidity in the air-conditioning cabinet. A gametocyte count was made on each case just before feeding the mosquitoes on them. The survivors were dissected after different intervals and their guts and glands examined. The detailed result is shown in Table I.

Table I. A. annularis.

Combination of temperature and humidity.	Percentage of infectivity with.			Longevity of A. annularis.
	M. T.	B. T.	Q.	
50°%F—50%	0%	0%	0%	62·7% (8—12 days).

Work on the infectivity of *A. culicifacies* was begun and is being efficiently conducted by Mr. L. B. Siddons. This species is probably the most widely distributed transmitting mosquito in this country, but cannot be obtained in sufficient numbers for experimental purposes within

130 miles of headquarters. The larvae have been collected mainly in Asansole (132 miles) and Dangoaposi (228 miles).

During the period 573 females were fed on 17 gametocyte carriers (B.T. 13; M.T. 2; Q. 1; and mixed B.T. and M.T. 1). The infectivity was studied, in more or less preliminary experiments, for the most part under combinations of temperatures between 80°F and 90°F and relative humidities between 40% and 95%, in the air-conditioning cabinet. The results are summarised in Table II.

Conjointly, control experiments have been performed under ordinary fluctuating conditions in the laboratory (Table III).

Studies on the effect of various antimalarial drugs were continued and reports on "Prontosil" and 'M3' by Col. Chopra and Dr. Basu have been published.

Table II. A. culicifacies under controlled conditions (air-conditioning cabinet).

Dry bulb Temp. (°F)	Relative Humidity (%)	Number of Mosquitoes Fed.	Parasite.	Number Dissected.	Glands Positive.
70	40	16	Mixed B. T. and M. T.	7	0
80	44	21	B. T.	0	—
79	65·6	62	Mixed B.T. and M. T.	9	0
80	80	47	B. T.	18	9
84	80	109	B. T.	5	0
84	95	18	M. T.	9	2
84	95	34	B. T.	18	6
87	55	26	B. T.	13	2

Table III. A culicifacies control experiments (laboratory conditions).

Mean Maximum Temperature (°F).	Mean Minimum Temperature (°F).	Mean Relative Humidity.	Number of Mosquitoes Fed.	Parasite.	Number Dissected.	Glands Positive.
89.1	83.0	84.7	49	Mixed B. T. and M. T.	14	1
			32	B. T.	11	0
91.0	78.3	76.3	53	B. T.	19	0
			23	Q.	8	0
94.5	79.7	70.6	83	B. T.	35	17

10. Blackwater fever enquiry under Dr. K. V. Krishnan, All-India Institute of Hygiene and Public Health, Calcutta.

With the demonstration of the therapeutic efficacy of cortin in blackwater fever, the necessity for a reliable method for the preparation of this hormone has been felt in this laboratory. Commercial preparations are usually found to be untrustworthy and in the case of preparations of established reputation such as those of the Wilson Laboratories, U. S. A., the cost is prohibitive and the supplies limited. Methods for the preparation of cortical extracts are indeed very numerous and, in the face of much disagreement about the relative advantages of these, it is difficult to make a choice from a mere perusal of the methods. Moreover, the general opinion is that the preparation of potent cortical extracts is by no means an easy task. This is because the methods for the preparation of the hormone involve long and laborious stages of empirical fractionation of the adrenal gland, and no means are available for determining the potency of the extracts at the different stages of fractionation. Only when the extract is quite free of proteins, fats and adrenaline can it be used for a biological assay, as otherwise the animals under test succumb to the toxic effects. The preparation of the cortical extract is really a separation of these toxic bodies by methods which do not inactivate the hormone. The different methods described in literature employ different procedures for this purpose. Satisfactory results have not been obtained from all the methods. The method of Cartland and Kuizenga with a few modifications appears to be the simplest and the best. The details of this method as modified are discussed below :

Collection of glands.

In the matter of collecting and preserving the glands before actual extraction, there seems to be no agreement between different workers. It is well known that the adrenals undergo rapid autolysis. In Swingle and Pfiffner's method the glands are packed in ordinary ice and extracted within 24 hours, after removal from the animals. Cartland and Kuizenga get their glands packed in dry ice. Grollmann observes that ill-preserved and consequently autolysed glands yield extracts of poor potency. He finds that with glands frozen in liquid air, dry ice and ordinary ice the amounts of the hormone obtained are in the ratio of 500 : 100 : 10, respectively. There is, however, a publication by Swingle and Pfiffner in which they say that autolysis does not reduce the potency of the extracts. In our experience, glands collected in ordinary ice do not yield good extracts. This is probably due to autolysis, for in ordinary ice the glands are very slow to cool. Twenty glands kept stirred in an aluminium tumbler surrounded by a freezing mixture of ice and salt took about 20 minutes to cool down to plus 10°C. In a tropical country like India it is all the more necessary to freeze the glands immediately. It is best to collect the glands in liquid air (-180°C) after a preliminary cooling in a mixture of dry ice and acetone (-80°C). The frozen glands are powdered and the extraction proceeded with. The intense freezing and thawing enables a thorough rupture of the cells, yielding thereby a more potent extract.

Methods for the removal of proteins.

The fact that cortin is soluble in fat solvents in which proteins do not dissolve has enabled all workers to eliminate these quite easily. The

gland is directly extracted by a fat solvent which takes up the cortin along with the fat and adrenaline. Hartmann and Brownell employ ether for this purpose while Swingle and Pfiffner use alcohol, while later workers like Kendall, Kutz, Grollmann, Cartland and their co-workers employ acetone. Ether being a non-miscible solvent, the extraction by it of the wet gland is not effective, and moreover, it often contains peroxides which have a destructive effect on the hormone. In these respects alcohol is better and acetone even superior; the latter takes up less of phospholipoids and gives weight for weight more potent extracts.

Removal of fats.

The cortical extract at this stage contains large quantities of adrenaline and neutral fats. The removal of both these bodies presents immense difficulties and the cortin is considerably inactivated or lost during these processes. If these impurities are not completely removed the extracts are useless for therapeutic purposes. Grollmann claims to have separated all the fat by freezing and filtering. This method was not found to be quite satisfactory. Swingle and Pfiffner separate the fat by repeated distribution of the extract between 70% alcohol and petrol ether. The process is very slow and provides many chances for the complete loss or inactivation of the cortical principle. Cartland and Kuizenga claim that if an aqueous acetone extract of the gland is first evaporated to remove the acetone and then shaken with petrol ether, the neutral fat alone is extracted. From our experiments it is believed that although petrol ether does not take up cortin from its pure aqeous solution, it does dissolve the hormone when it is mixed with fat. However, by restricting the extent of the petrol ether extraction it is possible to minimise the loss of cortin.

The removal of adrenaline from the extract.

This is also difficult because every method which removes adrenaline also removes or destroys some of the cortin. As early as 1931 Hartmann and Brownell remarked about the futility of attempting to remove adrenaline by methods involving washing of the ethereal solutions with alkali or acid or treating aqeous or alcoholic solutions with absorbents like alumina, kaolin, permutit, animal charcoal etc. According to these authors the adrenaline can be removed completely by these processes but much cortin is also removed. Swingle and Pfiffner employed therefore the dissected cortex during their earlier work. This process of dissecting out the medulla is slow and laborious and unless special precautions are taken, autolysis cannot be avoided. Swingle and Pfiffner, themselves, in their subsequent papers and all other later workers have given up this dissection procedure and introduce at the end of the extraction a step to remove the adrenaline. In one publication Swingle and Pfiffner recommend the alkali method, but realising that cortin is destroyed by alkali, they abandoned that process and made use of permutit adsorption. Hartmann and Brownell have criticised both the methods. In a modification by Kutz the alkali is replaced by sodium bicarbonate which gives a pH of about 7.0. In our experience the destruction of adrenaline by autoxidation at this pH is too slow to be of any practical value.

Nobody seems to have exploited the enzymic destruction of adrenaline by suitable oxidase preparations. These are being studied at present and the results are awaited with interest.

Cartland and Kuizenga have introduced yet another modification for the removal of adrenaline from cortin. Their claim is that ethylene dichloride takes up from aqeous solutions cortin, but not adrenaline. This claim is attractive; but in view of the fact that no evidence is available to show that this method has received universal acceptance, their claim had to be verified. On determining the partition co-efficient of adrenaline between water at pH 5.0 and this solvent it was found that the values were of the order of 500 : 1. This procedure has therefore been employed in our extraction. Our finding supplies the much needed confirmation of this useful method for separating adrenaline from cortin. The modified method of preparation of cortin that has been evolved is given below.

Beef adrenal glands are removed within ½ hour of the slaughter of the animals and as much of the fat as possible separated mechanically. The glands are then thrown into an enamelled or galvanised iron vessel containing a mixture of powdered dry ice and acetone. In this frozen condition they are brought to the laboratory and transferred into liquid air contained in a wide-mouthed "Thermos" food jar. The glands are frozen as hard as stone and are powdered in a mortar. Before a fine consistency is reached the material may require another freezing in liquid air and re-pounding. The powder is thrown into dry acetone kept in a shaking bottle and left at room temperature over-night. For one kilo of glands *i.e.*, about 200 beef adrenals, 2 litres of dry acetone are used. The next morning, the bottle is shaken for about half an hour on the machine and the acetone filtered through a Buchner funnel. The glandular matter is extracted twice again with 2 litres of 80% acetone each time and the combined extracts evaporated *in vacuo* under 35°C to remove the acetone. With a good filter pump and a water head of fifty feet and large ice-cooled condensing coils, about 50 mm. Hg. vacuum is obtained. By suitable arrangement the condensed acetone is removed at intervals effecting about 50% recovery of this costly solvent. This fact is important because in an ordinary vacuum distillation of acetone very little of the liquid is recovered.

When all the acetone is removed, the aqueous oily fluid is shaken with petrol ether, 50 cc. proportions twice and the ether layer separated. The aqueous layer is now essentially free from fat. It is now thoroughly extracted with ethylene dichloride and the ethylene dischloride layer separated and vacuum-distilled below 35°C. The solvent is recovered in this case also. A little syrup is left behind in the distillation apparatus which is taken up in 10 cc. of absolute alcohol and mixed with an equal volume of petrol ether. To the clear solution thus formed 3 ccs. of distilled water are added (1 cc. at a time in three stages). By so doing, more and more petrol ether separates without emulsification. The petrol ether removes the last traces of fat, and other inert material, while the 70% alcohol layer retains the cortical principle. The alcohol is removed by distillation *in vacuo* under 35°C and the product left behind is treated with about 5 cc. of normal saline and centrifuged at high speed when a little amount of tarry matter is deposited at the bottom of the tube. The supernatant clear fluid is diluted to 16 cc. with normal saline and sterilised by passage through a Jena glass bacterial filter.

An extract thus prepared is almost colourless, clear and free from proteins, fats and phospholipoids. It contains 0.01 m.gm. of adrenaline per cc. and a dose of 1 cc. given intraperitoneally to a mouse weighing 15 gms. does not produce any toxic symptoms. It is also found to be tepont by biological assay on bilaterally adrenalectomised mice.

TECHNIQUE OF ADRENALECTOMY.

Mice are shaven on the back just below the ribs and tied to a dissecting board. They are anaesthetised by a mixture of chloroform and ether and a longitudinal incision made below the ribs in the region of the kidney. The adrenals are then carefully exposed and removed by means of a pair of fine forceps without damaging the kidney. Care is taken to remove the whole of the gland and the accessory tissue. The kidney is allowed to take up its normal position and the incision sutured. Adrenalectomy on the right side is somewhat more difficult than on the left as the gland is hidden under one of the lobes of the liver, and any mechanical injury to the liver while exposing the adrenal results in the death of the animal. Operations on both the sides can be finished within 10 minutes. After a successful operation the animal feels fit and moves about within an hour.

One set (not less than 3) of adrenalectomised animals is given intraperitoneally 0.2 to 0.4 cc. of cortical extract (corresponding to 15-30 glands) per day while another set is kept untreated as control. A third set of animals is subjected to a sham operation in which everything except the actual removal of the adrenals is done. The animals function as controls for the mortality due to surgical shock. Usually in the third set no animal dies, while in the second set of untreated animals all die within 24 hours. A few of the group receiving cortin, at times die, but not always. A high percentage of cortin-treated animals survive for 5 or more days and die only when cortin is withheld.

11. Enquiry on the maxillary index of *A. stephensi* under Mr. R. Senior White, Malariologist, B. N. Railway, Calcutta.

The enquiry, which was closed down in April 1938 with the departure on leave of the Officer-in-charge, was resumed from Ferbuary 18th, 1939, and its continuation was sanctioned for the year 1939-40. The present note deals only with work accomplished in 8 1/2 months from February to October.

For some reason *A. stephensi* has become much rarer than it ever was before in Calcutta. During the period under review a total of only 77 specimens have been obtained. Two-thirds of these were obtained in the trap net baited with a cow. Unfortunately the house-holder who had so materially assisted the enquiry by permitting this from its inception withdrew his permission in August, and it has been impossible to find another suitable stable with an owner willing to co-operate, thus further reducing the supply of material for work. House by house collections were restricted to Wards IX-XI, which earlier experience had shown to provide most specimens, but even these wards later proved almost sterile of *stephensi*. From September the North end of the City, where proofing of cisterns, if this be the cause of the increasing scarcity, is less likely to have progressed than elsewhere, have been searched from September, but with little better result. The human bait trap net has not yielded a single specimen this year.

Not only has the supply of specimens thus been extremely low, but extreme difficulty has been experienced in making the captures oviposit for determination of the race of each individual. Quite half of the specimens have died without ovipositing, though every attempt has been made to re-feed them to this end. It has been found that the thin skin of the inguinal region of a dog is preferred as a feeding site to the human arm or the skin of a cow. Further, even when re-feeding has been repeatedly done, the gestation period has been extremely prolonged, in the extreme case 43 days. This difficulty was not experienced up to 1938, and no change has since been made in the method of conserving living specimens.

In the determination of race recourse has had to be made to infertile, malformed eggs, and even to eggs extracted from the abdomens of dead females. Such eggs are usually only measureable in respect of length, the floats not being distended, and in the case of unlaid eggs, there is some evidence that mosquito eggs swell after deposition in water. However, not allowing for this, of 49 hatches obtained 26 have belonged to race B and 23 to race M. Both races are therefore present in Calcutta, and thus, as adumbrated in my note presented in 1937, all work done on the maxillary index prior to December 1937 refers to the two races together, and cannot be used.

A great difference in fertility has been found between races B and M. In the former nearly 60% of layings hatched, in the latter about 17% only. This difference in fertility is difficult to correllate with the presence in the City of both races in approximately equal numbers.

Unless, which appears unlikely, very much larger numbers of specimens are obtained in the next five months, it will not be possible to obtain 200 counts of individual maxillae, (to make a difference of 1 tooth in the index significant) before the end of the financial year.

However, the number of specimens coming in is so small that a continuance of the enquiry for another year does not seem financially justified, and its discontinuance from the end of the year is suggested. The results, such as they are, will be written up in April in the hope that their publication will stimulate the Mosquito Control Department of the Corporation, who could, if they wished, carry out this work with specimens obtained in the course of their routine check catches, without special expenditure, to do so.

The Corporation's mosquito staff are alone in possession of data which may serve to elucidate why *A. stephensi* is apparently becoming more and more rare in the City.

12. Pharmacological enquiry on plasmoquine and atebrin under Dr. B. B. Dikshit at the Haffkine Institute, Bombay.

Pharmacological investigations on the two synthetic anti-malarial drugs, plasmoquine and atebrin were continued during the year 1939.

Investigations on plasmoquine were concerned with the destruction of the drug in the body and the isolation of the decomposed product from the body tissues. Experiments on atebrin chiefly dealt with the mode of action of the dye in malaria.

Plasmoquine.

Experiments made last year had indicated that plasmoquine is rapidly destroyed or converted into some other substance in the body Different organs of the body were therefore investigated to determine their relative power of destroying plasmoquine. Aqueous extracts of these organs were prepared after removing lipoid material by acetone and it was found that these aqueous extracts retain their power of destroying plasmoquine and exhibit it in the following order:—Brain > liver > spleen > testis > kidney. It was further observed that the destuction was maximum at a temperature of 40°C and no destruction of plasmoquine took place if it was kept in contact with the extracts at 0°C. Hydrochloric acid and cyanides inhibited the activity of the extracts while fluorides only diminished it. It appears therefore that the agent which destroys plasmoquine is of the nature of an enzyme.

Attempts were then made to isolate the decomposition product from the animal tissues. As the liver possesses the power of destroying plasmoquine to a considerable degree and as it is possible to maintain its physiological activity in an isolated condition for a period of several hours, this organ was selected for experiment. A technique was evolved by which a known volume of a saline medium containing plasmoquine could be circulated over and over again through the physiologically active liver continuously for a period of 5 hours. It was found that plasmoquine was destroyed rapidly by the prefused liver and most of the destroyed product was retained in the organ itself very little passing on into the perfusion fluid. The liver perfused in this way was therefore pulped and subjected to chemical extraction. The method of chemical extraction involved precipitation of proteins by trichloracetic acid, removal of the undecomposed plasmoquine by ether and recovery of the decomposed product as a phosphotungstate. This phosphotungstate of the decomposition product of plasmoquine is light yellow in colour insoluble in water and readily soluble in alkalis.

The pharmacological action of this decomposition product was studied to some extent. Toxicity tests showed that it is considerably less toxic than plasmoquine itself. Thus *Paramaecia caudatum* can survive in a concentration of 1-10,000 for 24 hours while this concentration of plasmoquine kills them in 5 minutes. Cats can tolerate a dose of 10 mg/kg which is about 3 times the tolerated dose of plasmoquine. The marked irregularities of the heart which are a constant feature of moderate doses (2 mg/kg) of plasmoquine are absent after even large doses (20 mg/kg) of this decomposition product. The depressant action on the circulatory and respiratory systems is less marked than that of plasmoquine. The movements of the gut and its tone are increased while they are decreased by plasmoquine. The only action which was manifested by this decomposition product approximately to the same extent as that by plasmoquine was that of methaemoglobin formation.

Atebrin.

Investigations on atebrin were continued and were chiefly concerned with the mode of action of this dye in monkey malaria. In order to see

the possibility of a direct lethal action on the malarial parasites (*Plasmodium knowelsii*) *in vitro* experiments with defibrinated blood of infected monkeys were made. It was found that the parasites were not killed by a concentration of 1-200,000 of atebrin acting for 24 hours at room temperature; with a concentration of 1-100,000 of atebrin acting for 24 hours at room temperature, the parasites were usually killed and only occasionally were they found to survive. This concentration of 1-100,000 however failed to kill the parasites at room temperature or at 37°C in 6 hours. Estimations of atebrin concentrations in blood were then made and it was found that the maximum concentration the mammalian heart could tolerate was in the neighbourhood of 1-90,000. It was also observed that the concentration of atebrin in blood after administration of largely varying doses in experimental animals found a threshold level of 1-300,000. When larger doses were injected the concentration went up to 1-200,000 or even higher but came down to the threshold level in a few minutes. After administration of doses equivalent to therapeutic doses in man, the blood concentration of atebrin varied between 1-300,000 to 1-500,000. These experiments showed that the lethal concentration of atebrin for the *Plasmodium knowelsii* was about 1-100,000 and the usual concentration that was present in blood after curative doses of atebrin was in the neighbourhood of 1-300,000. It therefore appeared that atebrin may not exert a direct lethal action on *Plasmodium knowelsii*.

Attempts were therefore made to see if the reticulo-endothelial system played any part in the manifestation of therapeutic action of atebrin. Splenectomized monkeys were infected with *Plasmodium knowelsii* and it was found that the usual curative dose of atebrin was able to exert its action in splenectomized animals also. Large doses of Indian ink were then injected in infected monkeys and again the same dose of atebrin produced its full curative effect. In some experiments with splenectomized and ink blocked monkeys however the usual dose of atebrin failed to bring about a complete cure. There experiments therefore show that the reticulo-endothelial system plays some part in the mode of action of atebrin.

NUTRITION.

13. Nutrition researches under Dr. W. R. Aykroyd at Coonoor.

Satisfactory progress has been made during the year with the schemes of research outlined by the Nutrition Advisory Committee, and new ground has been broken in various directions. The lines of investigation followed included the following :

The composition of foodstuffs.

Jansen's thiochrome method for estimating vitamin B_1 has been adapted for application to cereals. By this means, the vitamin B_1 content of a series of rice samples at different stages of milling has been determined and the effect of parboiling, washing and cooking on the vitamin worked out in detail. The following figures illustrate the effect of milling on the vitamin B_1 content of raw rice.

	Vitamin B_1 (parts per million).
Rice (shelled only)	4.0
Rice (once polished)	1.8
Rice (twice ,,)	1.0
Rice (thrice ,,)	0.7

When rice is previously parboiled, the effect of milling is different. During the process of steaming, some of the vitamin B_1 contained in the germ and pericarp diffuses through the grain and cannot be removed when milling subsequently takes place. Whereas the vitamin B_1 value of raw rice on milling may sink to 1.0 parts per million or below, that of milled parboiled rice usually remains above 2.0.

Certain workers in America—Cowgill, Williams and Spies—have demonstrated that the ratio vitamin B_1/calorific value can be used to predict the beriberi-producing effect of diets. If the ratio is below 0.250, there is danger of beriberi. Applying this criterion to typical Indian rice diets, it is found that the ratio may fall below 0.250 when the staple is raw milled rice, whereas it usually lies above this point when the diet is based on parboiled rice, whether home-pounded or milled, or home-pounded raw rice. About 600 microgrammes daily represent the intake of vitamin B_1 necessary to prevent beriberi when the calorific value of the diet lies within the usual range. Typical Indian diets based on parboiled rice supply 700-900 microgrammes daily, whereas the quantity supplied by milled raw rice diet is often below this figure. These facts can be correlated with the geographical incidence of beriberi in India. Beriberi is a serious public health problem only in the Northern Circars district of the Madras Presidency. The explanation is that in this area the population prefers raw (unsteamed) rice, whereas in nearly all the other rice-eating areas of India, parboiled rice is consumed by the mass of the population.

Washing and cooking : Many of the food factors present in rice are readily soluble in water and losses occur in the preparation of rice, the extent of which depends on the amount of water used for washing, the number of washings and whether the cooking water is discarded or not. The losses are of the following order : calories, 15% ; protein, 10% ; iron, 75% ; calcium and phosphorus, 50%. The loss in the case of vitamin B_1 and nicotinic acid is of the order of 40-50 per cent. It is the first

washing which causes most of the loss and much more vitamin B_1 passes into the wash water than into the conjee. These facts are of considerable practical importance.

The nicotinic acid content of a large number of foodstuffs has been studied by the cyanogen bromide method evolved in the Laboratories. It has been found that nicotinic acid is distributed in the rice grain in the same way as vitamin B_1 and diffuses through the grain on parboiling. Special attention has been given to the nicotinic acid content of typical rice and maize diets with the object of clarifying the etiology of pellagra, which is largely a disease of maize-eaters. The striking therapeutic effect of nicotinic acid on pellagra is now fully established. Typical Indian rice diets may, however, contain less nicotinic acid than maize diets known to give rise to pellagra, and the reason why the rice-eater rarely develops classical pellagra remains obscure. The observation that typical rice diets have a low nicotinic acid content may have clinical applications.

Chemical methods for the estimation of vitamin B_6 are being investigated and correlated with the results of biological tests. Work on foodstuffs has included the determination of the vitamin A content of numerous samples of fish liver oils in collaboration with the Director of Fisheries, Madras. Shark liver oil has been found to be exceptionally potent, and this substance has considerable possibilities as a cheap source of vitamin A. The vitamin C content of the Indian gooseberry, a very rich source of this vitamin, has been extensively studied by chemical and biological methods, special attention being given to the effect of storage and various methods of preparation and conservation. In addition, the routine investigation of the chemical composition of foodstuffs not hitherto analysed has been continued.

Various animal experiments.

Some 60 monkeys have been under experiment, the purpose of the investigation being to study the effect on health of long-continued feeding on deficient diets resembling those consumed by the poorer classes in India. The deficiently-fed groups, as compared with a control group on a well-balanced diet, have shown a high mortality and an interesting and varied pathology. The results throw light on the relation between diet and various intestinal conditions. In collaboration with Major Mulligan, Director, Pasteur Institute of Southern India, Coonoor, the relation between diet and immunological reactions in experimental malaria is being studied. Normal haematological standards for the monkey *Macacus sinicus* have been established.

Experiments on rats have shown that this animal can synthesise nicotinic acid. This finding explains why rats are able to thrive on diets which produce pellagra in human beings, whereas dogs, pigs and monkeys on similar diets develop pathological conditions due to nicotinic acid deficiency. The nicotinic acid content of various organs in rats and monkeys has been investigated.

The discovery that excess of fluorine in drinking water may cause "mottled enamel" in teeth and certain other bone changes has aroused considerable interest. It appears that fluorine poisoning is of considerable public health importance in India. Experiments with rats have been undertaken to study the relation between fluorine intoxication and other factors in the diet. It has been found that calcium has a remarkable effect

in mitigating the toxic effects of fluorine. Animals given sub-lethal doses of fluorine survived in fair condition for 6 months or more when they received 0.7 per cent calcium in the diet representing an adequate intake of this element. On the same diet, containing a similar amount of fluorine but less than 0.1 per cent calcium, they died within 15 days. The addition of increasing doses of calcium to experimental diets containing fluorine correspondingly prolonged the survival period. Progressively decreasing doses of fluorine given with a diet almost free from calcium had the same effect. That the calcium contained in natural foodstuffs has a protective action was shown by experiments in which ragi (*Eleusine coracana*) and rice, cereals which are respectively rich and poor in calcium, were substituted for starch in the synthetic experimental diet. The relation between fluorine intoxication and calcium and phosphorus metabolism is being further investigated.

Pathology.

An investigation of the relation between diet and pathological changes in the stomach and duodenum in man and experimental animals was carried out in collaboration with Dr. Ian M. Orr, with the object of throwing light on the etiology of peptic ulcer in India. The results indicate that changes in the mucous membrane and Auerbach's plexus occur as a result of diet deficiency, and suggest that such changes may be of importance in the causation of peptic ulcer. Varying degrees of degeneration of the myelin sheaths of the spinal nerves produced in young animals by diets deficient in vitamin A and carotene have been previously described. A study has been made of the effect on rabbits of adding carotene in adequate amounts to the deficient diet, after the animals had developed typical clinical signs of vitamin A deficiency. It was found that, while growth was restored and recovery from xerophthalmia took place, the anatomic lesions in the peripheral nervous system persisted and showed no evidence of regeneration.

Other pathological work included the investigation of the effect of various kinds of diet on the parathyroid gland, and the detailed study of lesions occurring in deficientlyfed monkeys.

Various biochemical investigations.

The excretion of nicotinic acid by human beings under normal circumstances and the excretory response to test doses have been found to vary with the state of nutrition and dietary habits of the individual. The excretion of nicotinic acid in these circumstances by patients suffering from various deficiency diseases, and by ill-nourished hospital patients, is lower than that of normal controls. The excretion of vitamin B_1 by individuals consuming various diets has also been studied, with the ultimate object of establishing an optimum standard of vitamin B_1 intake.

It has been shown that vitamin C protects certain enzymes (phosphatase and amylase) against destruction by ultra-violet light. This may be due to the fact that the vitamin filters off the wavelengths which are lethal to the enzymes.

Various other lines of biochemical research have been followed.

Field work.

(*a*) *Diet surveys* : Knowledge of the dietary habits of groups in various parts of India has been extended. Surveys of more than 50 groups have

now been made. A great part of this work has been carried out by field workers associated with the Laboratories, but mention must be made of the fact that useful investigations have been conducted in certain areas by Provincial and State officers who have taken the training course in nutrition in Coonoor. One important observation, which simplifies the approach to the practical nutrition problems, is that the poor rice-eater's diet is very uniform all over India. The surveys clearly indicate the defects of Indian diets and the changes which are necessary to improve them. The results obtained should therefore be of value in formulating agricultural policies.

The following figures show the foodstuff composition of the diet consumed by rice-eating groups studied in various parts of India.

Mean daily intake of various foodstuffs.

(Ozs. per adult man daily)

	Madras (Rural area)	Madras (Urban area)	Bengal (Rural area)	Assam (Rural area)	Orissa (Rural area)	Central Provinces (Rural area)	Madras (Tea plantation labourers)
Rice	15.0 (millets 2.0)	14.2	25.0	11.0	19.0	26.0	18.0
Pulses	0.5	0.9	0.4	0.9	1.0	1.1	1.0
Leafy vegetables ..	0.3	0.6	0.2	0.8	1.4	1.5	none
Non-leafy vegetables	0.9	2.8	7.0	4.0	6.0	3.0	3.0
Vegetable fats and oils	0.1	0.5	0.3	0.3	0.3	0.2	0.5
Fish, meat and eggs	0.8	1.5	0.7	0.7	0.6	negligible	1.5

Surveys have been made of families showing a high incidence of leprosy with the object of throwing light on the relation between diet and the incidence of this disease. In the majority of surveys the state of nutrition of children in the area concerned has been simultaneously investigated.

(*b*) About 4,000 school children in the Nilgiris, Coimbatore, and Kurnool districts were examined for dental caries. Their ages ranged from 5 to 18 years. About 55 per cent of the children had one or more carious teeth. Out of 107,821 teeth examined about 5% were carious. The incidence of caries was higher in the deciduous teeth than in the permanent teeth. Children living in rural and urban areas on a diet largely composed of cholam (*Sorghum vulgare*) showed less caries than rice-eating children in urban areas. The incidence appears to increase with a rise in economic status, the poorest children having the soundest teeth. No difference was observed in the frequency of caries in boys and girls. In general, caries is considerably less common in Indian children than in European and American children.

(*c*) An association between "mottled enamel" in teeth and excess of fluorine in drinking water has been established by workers in various countries. It has been suggested that there may be an inverse relationship between the incidence of caries and "mottled enamel". This was not,

however, borne out by investigations on school children in various parts of India. Of 832 children living in an area in which fluorosis is endemic and showing "mottled enamel", 46 per cent were free from dental caries, and the percentage of all teeth found carious was 5.6. In another area free from fluorosis 677 children were examined; 55 per cent were free from caries and the total caries incidence was 5.1 per cent. Dietary and other conditions in the two groups were approximately the same.

(*d*) The response of cases of stomatitis, and conditions associated with it, to treatment with nicotinic acid has been further studied. The results are not consistent; some cases respond with alacrity; others show little improvement. The explanation appears to be that deficiency of other members of the B_2 group of vitamins is also concerned in the etiology of these conditions.

(*e*) Pathological amounts of porphyrin in the urine have been demonstrated in certain cases of pellagra. This is not, however, an invariable accompaniment of the disease. The urinary porphyrin excretion of persons living on a poor rice diet with a low nicotinic acid intake, and showing signs of stomatitis and glossitis attributable to deficient intake of the B_2 group of vitamins, has been investigated. It was found to be within normal limits—*i.e.*, below 50 mircrogrammes per day.

The rice problem.

Considerable attention has been given to this question throughout the year. It has proved possible to combine the results of diet surveys and laboratory investigations into a composite picture. A memoir entitled "The Rice Problem in India", which deals with many different aspects of the subject, is being published. This work was undertaken in accordance with the recommendations of the League of Nations Inter-Governmental Conference on Rural Hygiene, 1937 and the Nutrition Advisory Committee, I. R. F. A. (1937 meeting).

Training course for health officers.

Seventeen workers from various Provinces and States in India attended a course which was held during the months, April-June. A medical officer from Siam was included among the pupils. This is the third course which has been held, and sufficient experience has now been gained to ensure that it should run smoothly and involve a minimum of effort to the staff. A number of workers attending previous classes are now employed as full time "nutrition officers".

Education and propaganda.

The usual activities have been continued. A new edition of Health Bulletin No. 23 has been issued during the year. There are many indications that elementary knowledge of nutrition is becoming widely disseminated in India. A nutrition museum has been established in the Laboratories which includes numerous photographs and exhibits.

Collaboration with the Technical Commission on Nutrition of the League of Nations.

A series of notes was prepared and forwarded to Geneva in connection with the agenda of a meeting of the Technical Commission on Nutrition

which was to have been held in Geneva from August 28th to September 1st, 1939. Contact was established with workers in Ceylon, Malaya, the Netherlands Indies, Siam, the Philippines and China, and material received from these countries was incorporated in the data sent to Geneva. Owing to the war, however, the meeting of the Technical Commission did not take place. Should circumstances permit the Technical Commission to carry on with its work, the Laboratories will continue the task of collaboration.

14. Additional nutrition enquiry under the Liaison Officer at the Imperial Agricultural Research Institute, New Delhi.

The Liaison Officer followed the lines of activities of the previous years, in addition to some newer lines of work which may be mentioned under the following heads :—

(1) Collection of evidence from medical authorities and public health workers regarding the state of nutrition of the villagers and townspeople.

It appears from response from various sources that no diet or nutrition surveys have yet been taken up in the British Indian provinces except Bengal, the Punjab and Bihar, Burma and one or two Indian States.

Many of the Health Officers, who underwent a course of training in nutrition at Coonoor, regretted that they were not doing any nutrition work while some were hoping to take to it as soon as they received instruction from their respective authorities.

(2) Liaison between human nutrition and agricultural departments.

(*a*) The Liaison Officer discussed with the Agricultural Commissioner the possibilities of improving nutrition by propaganda through the Imperial Council of Agricultural Research and Provincial Agricultural Departments, by attempting, through discussion and persuasion, to arrange for an experimental alteration of village agriculture by bringing in more milk, more fresh leafy vegetables, more fruits, more pulses (if necessary), and by increased production of cereals (if necessary), in selected suitable villages, the scheme being extended later on to other villages. He also discussed the question of encouragement of the growth and wider use of pulses in Indian diets.

(*b*) The Liaison Officer made an extensive tour in Bengal to gather information regarding the health and nutrition of the people of the province. This study revealed a regrettable amount of deficiency of vitamins A and C, of minerals like calcium and iron, and of proteins and fats of animal origin existing all over the province. The Director of Agriculture has been informed about the results and requested to attempt to remedy this by suitable crop-planning and necessary agricultural re-adjustments.

The Liaison Officer also toured in Bombay, Mysore, Sind, the Punjab and North-Western Frontier Province with a view to gaining first hand information regarding the economics of agricultural and nutritional problems.

(3) INVESTIGATION OF THE VARIATION IN MINERAL CONTENT OF FOODSTUFFS GROWN IN DIFFERENT AREAS UNDER VARYING CONDITIONS OF SOIL AND CLIMATE.

Samples of rice from different areas of U. P. (Nagina), Bihar (Ranchi), Assam (Jorhat and Habiganj) have been analysed and a good deal of variation detected in important constituents like protein, calcium, phosphorus and iron.

(4) THE AMINOACID CONTENTS OF THE COMMON INDIAN PULSES AND OF SOME VARIETIES OF WHEAT.

These have been investigated.

(5) INVESTIGATION OF THE NUTRITIVE VALUE OF MILK FROM AN UNCOVERED HEIFER.

Feeding experiments were undertaken on rats as well as human subjects, and it has been found that this particular milk was in no way inferior in nutritive value to a sample of ordinary milk from a mixed stock. Chemical analysis however showed that the uncovered heifer's milk was richer in total solids, total protein, phosphorus and iron contents, with less casein than ordinary milk. The carbohydrate, calcium and globulin contents were almost equal in both the cases.

(6) INVESTIGATION OF SUITABLE SUPPLEMENTS TO COUNTERACT THE TOXIC EFFECTS OF A HIGH PULSE DIET WITH CEREALS.

Yeast, butter and cod-liver oil have been found as effective agents for counteracting the toxic effects of high pulse diet with rice (the pulses being the five most common varieties such as lentils, Bengal gram, red gram, green gram and black gram), reported in the previous year's report. Vegetable oils like mustard or coconut oil, autoclaved yeast, and also carrots, and green leafy vegetables with equal amounts of carotene content equivalent to the amount of butter or cod-liver oil that was found effective as a detoxicant of the basal diet, were useless as supplements to prevent mortality and ill health characterised by loss of fur and stiffness of neck, etc.

(7) HEALTH BULLETIN NO. 23 (SECOND EDITION).

The Hindi synonyms of various foodstuffs mentioned in the Bulletin have been collected for inclusion in the second edition.

(8) A REVIEW OF THE LITERATURE ON THE NUTRITIVE VALUE OF PULSES.

This has been published in the Indian Journal of Agricultural Science, Vol. IX Part I, February 1939.

15. Enquiry on cheap well balanced diets under the Bombay Presidency Baby and Health Week Association.

Further investigations on balanced diets are being continued on the lines mentioned in last year's note. The investigation is being carried out along two lines, (I) nutritional and (II) analytical.

I. Nutritional.

Experiments with balanced diet No. III came to an end in December 1938. In this diet 9 ozs., of milk replaced the skim milk powder and the butter in balanced diet No. II and dal (Cajanus indicus) replaced the soya beans in balanced diet No. II.

Along with the above experiments soya beans were tried in another group of boys in which 4 ozs., of dal of the Reformatory diet (control diet) was substituted by soya beans. A separate control was kept for this experiment. The ideal diet of 1937 was discontinued and this group of boys was given the Reformatory diet in 1938 in order to observe the effects of the return to the Reformatory diet on the growth of the boys.

All the above experiments came to an end in December 1938.

The work during the year 1939 was confined to one experiment only as mentioned in the note on the nutrition enquiry submitted to the 16th Medical Research Conference, New Delhi, in December 1938.

In this experiment two sets of boys of the age groups 13 and 14 only have been taken. The routine Reformatory diet which served as a control previously was made vegetarian, and meat and other non-vegetarian substances in the previous control diet were replaced by an equivalent amount of vegetarian food substances.

The new experimental diet—balanced diet No. IV—contains 6 ozs. of milk added to the diet explained in the above paragraph and an equivalent quantity of pulses and oils added to the diet to serve as a control diet. This experiment was started in June 1939 and will be continued till the end of May 1940. The idea underlying the experiment as mentioned in our proposal is to find out the minimum quantity of milk which would improve an otherwise cheap but fairly well balanced vegetarian diet. The diet tables have been already submitted.

The above experiment is being carried out in the David Sassoon Industrial Reformatory School under the same conditions as those of last year. The nature of supervision and the precautions taken were the same as in the previous years.

II. Analytical.

Chemical analyses of fresh & dried fish:—During the course of the year analyses of fresh fish, dried fish and the estimation of vitamin A content of local fish liver oils have been completed.

Samples of fish were analysed for their protein, fat, calcium, phosphorus and iron contents. Vitamin A was estimated by antimony trichloride method using a Lovibond tintometer. The livers of the dried fish are not available for the determination of vitamin A since they are rejected before drying. The body oils of the dried fish do not contain any vitamin A.

The results show that dried fish is a cheap source of animal protein, calcium, phosphorus and iron.

Vitamin A content of fresh liver oils :—While the body oils of the fresh fish do not contain any vitamin A, the liver oils supply ample vitamin A. The vitamin A content of liver oils of some of the fresh fish is in some cases hundred times more than that of ordinary cod-liver oil. The results are shown in the following table :—

Scientific name	English name	Average weight of liver in grams	Average weight of oil in grams.	Vitamin A content in blue units per gram of oil
Stromateus.Niger	Black pomfret	123	2.59	52,477
Chirogentrus	Karli	94	11.59	5,194
Lates-Calgarifer	Cock-up	137	9.21	8,374
Polynemus-Tetratylus	Ravas	60	1.27	99,265
Sciaena-Bleekery	Ravas	233	12.91	9,494
Scoliodon-Sorrakewah	Sharks or dog fish	820	50.00	6,780
Ariusdussumieri	Cat-fish	210	18.25	378
Trichiurus-Savala	Ribbon-fish	55	8.40	839
Cybium-Kuhlil	Seer-fish	90	3.10	1,418
Macrones-Gluilo	..	110	32.02	6,403
Hangoli (Local name)	..	115	7.73	15,570
Mandeli (-do-)	..	105	3.49	29,967
Dhumi (-do-)	..	51	6.75	1,717
Sciaena-Miles	Indian salmon	180	15.90	6,532
Dasybatus-Imbricatus	Sting-ray	595	21.35	538

Vitamin A content of cod-liver oil (local sample) is 118 blue units per grm.

An investigation into the biological values of proteins of parched grain is being carried out in order to find out whether such parching affects the nutritive value of these food-stuffs. Parched grains are commonly used in various parts of India.

Experiments on growth tests on albino rats with diets containing ground-nuts, soya beans, etc., will be soon undertaken.

16. Enquiry into the basal metabolism of adults and children under Professor S. P. Niyogi at the Seth G. S. Medical College, Bombay.

During the year under review experiments for establishing standards for basal metabolism in normal children were undertaken. The subjects chosen were boys from the David Sasoon Industrial School, Matunga, Bombay. A total of 30 subjects was put through the tests. The tests were carried out under standardised conditions reported previously.

The data for the averages of age, vital capacity, pelidisi, oxygen consumption, etc. are given in the following table. The average oxygen consumption for the group was 170 c.c. per minute, the maximum individual reading being 203 c.c. and the minimum being 137 c.c. per minute. The average deviation from the Mayo-Clinic standards is -14.1%, from the Harris and Benedict standards -2.8% and that from DuBois standards -14.0%.

Table I.

	Age in years and months	O_2 consumption per minute.		% deviation	Pelidisi.	Vital capacity
		Actual	Mayo-Clinic Standard			
Average	13/9	170	199	— 14.1	92.2	1721
Maximum	16/1	203	223	0.0	100.9	2400
Minimum	12/2	137	172	— 23.8	88.2	1100

Other physiological measurements were recorded and the pulse, respiration and blood pressure were taken as indices regarding the state of health of the subjects during the experiments.

Further work on adults is also being continued. Several characteristics of locality are regarded as potential factors affecting basal metabolism. Environmental temperature and humidity may play an important role. With a view to finding out if these singly or together, would exert any influence on the basal metabolic rate 27 female subjects have been selected and each of them is being observed once a month and the observations will be continued for a year. For the sake of uniformity in the comparison of the data, the tests are being done in the non-menstrual period between the 4th and 10th day after menstruation.

The basal metabolism of adult men and women in Bombay has been found to be lower than that of the races inhabiting the temperate regions. The possible factors responsible for the lower rate of basal metabolism are also being investigated. It has been observed by some workers that the nitrogen metabolism of adults in Bombay is very much lower than that observed in Europeans and Americans. Experiments mainly conducted in America are also on record which show that basal metabolism might be related to the endogenous nitrogen metabolism of an individual. Experiments have therefore been undertaken to find out if there exists any relationship between basal metabolism and the endogenous as well as

exogenous nitrogen metabolism and whether it is possible to find in them an explanation of the lower basal metabolism of Indians.

Observations on ten men have been completed. The total nitrogen excretion during 24 hours in these subjects has been found to vary between 5 and 9 grams. Their protein intake was increased by supplementing their usual diet by a half to three quarter pound of meat daily. An increase in the daily nitrogen output followed. Weekly observations of the basal metabolic rate were taken extending up to four weeks. Urinary nitrogen was determined also at weekly intervals.

Average values of relevent observations on ten men put through the tests are given in the Table II.

Table II.

PRE-EXPERIMENTAL PERIOD.				EXPERIMENTAL PERIOD.		
Preformed Creatinine in urine in 24 hours.	Creatinine Co-efficient. (mg. creatinine weight in Kg.)	Total urinary nitrogen in 24 hours.	Av. B. M. R. (Cals per sq. m. per hour.)	Amount of extra protein fed per day.	Total urinary nitrogen on extra protein.	Av. B. M. R. (Cals. per sq. m. per hour.)
1219 mg.	23.2	6.95 gms.	33.07	1st week 39 gms.	8.51 gms.	33.5
				2nd week 43 ,,	8.99 ,,	33.2
				3rd week 46 ,,	9.11 ,,	33.1
				4th week 43 ,,	8.99 ,,	33.2

17. Ascorbic acid enquiry under Prof. B. C. Guha, at the University College of Science and Technology, Calcutta.

Vitamin C and toxins.

The previous work on vitamin C and toxins has been extended to the study of the effect of diphtheria toxin on vitamin C metabolism. A decrease in the free ascorbic acid content of the blood, adrenal, liver and kidney of the guinea-pig was observed after the injection of 0.5 m.l.d. of diphtheria toxin. The maximum depletion of vitamin C was observed in the adrenal gland, which suffered a loss of about 50 per cent as compared with that of normals, and there is also a definite reduction of the ascorbic acid content of the liver and kidney tissues after toxin injection. Moreover, it has been observed that, during the period of intoxication (caused by the injection of 0.5 m.l.d. of diphtheria toxin) there is a decrease in the free ascorbic acid excretion and an increase in the combined ascorbic acid (released after hot H_2S treatment) in the urine when compared with the

urinary excretion of ascorbic acid, both free and combined, of the same guinea-pig in the normal state of health. Similar behaviour has also been observed with tetanus toxin injection. These observations appear to support the view that vitamin C is of major importance in the detoxication process. Further work on the function of vitamin C in the detoxication mechanism is in progress.

Vitamin C and fungi and bacteria.

Previous work had shown that vitamin C had a stimulating effect on the growth and respiration of certain fungi but an inhibitory effect on certain bacteria grown in an artificial medium. This work has been extended to include other micro-organisms like *B. typhosus, B. diphtheria, Staph. aureus* and *Streptococcus haemolyticus.* The effect of vitamin C on these bacteria is also inhibitory in a dilution of 1 in 20,000.

These results naturally suggested the possibility that vitamin C might have some beneficial effect when animals were infected with live cultures of certain pathogenic bacteria. Preliminary experiments with *B. dysentericus Shiga* have given rather striking results. Guinea-pigs infected with the culture died within 48 hours, but those which were receiving extra doses of vitamin C during the fortnight previous to being infected survived. This work on the role of vitamin C in artificial infections with live cultures is in progress.

The intradermal test as an index of vitamin C nutrition.

In order to test the accuracy of the intradermal test for the assessment of the vitamin C status of the body, experiments were carried out on guinea-pigs, receiving a scorbutic diet, and their progressive rate of dye decolorisation was plotted against their body weight curves, which showed that the rate of decolorisation increased approximately at the same rate as the body weight diminished. The two curves agreed closely. Similarly when normal diet or ascorbic acid was given to the scorbutic guinea-pig, the decolorisation time gradually diminished and the body weight gradually rose agreeing fairly with the amount of ascrobic acid administered. While the rate of decolorisation and the amount of ascorbic acid administered, when given in graded doses to different animals, did not show an exact quantitave correlation, the general correlation observed appears to point to the reliability of the intradermal test within limits. A large number of young men, mainly students, have been subjected to the test and it has been found that the decolorisation time varies from 5 to 8 minutes.

Comparing the intradermal test with the ordinary saturation test, it has been found in general that the administration of a massive dose of ascorbic acid to human subjects produces a peak in their urinary excretion sooner than it reduces the decolorisation time in the skin test, and it appears that the urinary saturation test may not be a correct test for determining the vitamin C saturation of the body. Further work is in progress to investigate the relative merits of the two methods.

Ascorbigen or combined ascorbic acid in plant tissues.

Evidence has been furnished about the existence of ascorbigen in plant and animal tissues. It has been shown that ascorbigen can be extracted from dried cabbage by chloroform extraction. From the chloroform extract, ascorbigen can be further extracted with water. The aqueous

extract does not give indophenol titre, but on heat treatment in CO_2 atmosphere for 10 minutes appreciable reduction in dye value can be obtained. Later it has been found that about 70% of this reducing material is identical with ascorbic acid as determined by the action of the enzyme ascorbic acid oxidase at pH 5.6 at 40° for ½ hour.

Biological experiments have been carried out with scorbutic guinea-pigs and it has been found that similar types of growth curve are obtained when guinea-pigs are fed with synthetic ascorbic acid and ascorbigen preparations containing equivalent amounts of ascorbic acid (estimated titrimetrically).

Further concentration of ascorbigen from fresh cabbage juice by methods of adsorption has been attempted. Among the different adsorbing materials tried, active charcoal has proved to be the most effective. The ascorbigen can be eluted by heating the charcoal-adsorbate with a mixture of chloroform and absolute alcohol. On evaporation of the solvents, a very concentrated preparation of ascorbigen was obtained. It gives a strong Molisch test and contains about 11-13 per cent N. It was found to be non-dialysable. Further work on its purification and chemical nature is in progress.

The estimation of vitamin C in food-stuffs.

Based on the investigations on the ascorbic acid and ascorbigen of foods-tuffs, a method has been devised for the estimation of "total, true" ascorbic acid in food-stuffs by suspending the finely ground material in water and passing H_2S in hot condition for 15 minutes. After the removal of H_2S, the vitamin C value in one aliquot was estimated by the usual titration technique. Another aliquot was subjected to the action of ascorbic acid oxidase and then titrated against the dye, in order to eliminate non-specific reducing substances. The difference in the titration value of the two aliquots gave the value for "total, true" ascorbic acid. As in this method, the oxidase would be required as a routine reagent, its stability under different conditions of storage has been investigated. Biological experiments are in progress in order to verify biologically the values obtained by the above method.

The stability of vitamin C in a number of food-stuffs has also been investigated by this method.

The changes of blood-lipoids of normally fed and vitamin C deficient guinea-pigs.

In vitamin C deficient guinea-pigs there is a rise of the cholesterol content of blood, the average rise being of the order of 50 per cent as compared with that of controls. The total lipoid content of the blood also becomes higher but the total fatty acid content does not seem to suffer any significant change. This apparent relation between vitamin C and cholesterol metabolism of the blood is under further study.

18. Studies in human metabolism under Dr. K. P. Basu at the Dacca University.

In this enquiry the problem of nutrition is being tackled by direct metabolic experiments on human subjects. In order to formulate cheap ideal diets, knowledge regarding the requirements of the nutritionally essential elements like nitrogen, calcium, phosphorus, magnesium, iron and manganese by Indians, and the capacity of the typical Indian dietaries to supply these requirements is essential. A study on these lines would show whether the diets in common use reveal deficiencies of any of these elements and, if they exist in the dietaries, such a study would point to lines along which attempts are to be made to make good these deficiencies by suitable modifications and supplements.

Experimental diets.

Three diets were chosen (1) a non-vegetarian rice diet containing fish and pulses commonly taken by the people of Bengal and Assam and certain parts of South India (2) a vegetarian rice diet similar to the former one, only fish being replaced by more pulses consumed by the people of Orissa, Madras, Mysore and other parts of South India and (3) vegetarian atta diet containing pulse, taken by the people of the U. P., the Punjab, and other parts of Western and Central India. All the diets contained, besides the cereal and pulse, other food ingredients as indicated in Table I and were adequate with respect to calories. Experiments were performed on different subjects with these diets alone and also with the diets supplemented by 10 oz. of cow's milk.

Diet Chart.

Food-stuffs.	Diet I.	Diet. II.	Diet III.
Rice	600 g. (21.2 oz.)	600 g. (21.2 oz.)	Nil.
Atta	Nil.	Nil.	550 g. (19.3 oz.)
Dal (Pulse)	60 g. (2.1 oz.)	100 g. (3.5 oz.)	90 g. (3.2 „)
Fish	70 „ (2.4 „)	Nil.	Nil.
Vegetables	200 „ (7 „)	200 g. (7 oz.)	180 g. (6.3 oz.)
Mustard oil	30 „ (1.1 „)	30 „ (1.1 „)	Nil.
Ghee (Butter fat) ..	Nil.	Nil.	30 g. (1.1 oz.)
Sugar	50 g. (1.7 oz.)	50 g. (1.7 oz.)	50 g. (1.7 „)

Proteins.

The minimum nitrogen expenditure :—The minimum daily elimination of total nitrogen in urine and faeces combined, by subjects living on protein free diets were 2.880 g. (P.C.D.), 3.435 g. (S. N. D.) and 3.270 g. (G. C. N.). Obviously, these values represent the minimum metabolic activity of nitrogen in the subjects concerned. Hence the smallest amount of protein, that would have been theoretically necessary to make good these endogenous losses, would be 18.0 g. (P. C. D.), 21.5 g. (S. N. D.) and 20.4 g. (G. C. N.)

with a mean value of 20 g. per man-value or 27 g. per adult of 70 kilo body-weight.

Protein requirement for balance :—It might be pointed out here that the above replacement of nitrogen does not take place quantitatively in practice. The average minimum protein requirements for maintenance per 70 kilo-body-weight on rice diets, containing rice, pulse and vegetables, were 37.5 g. (G. C. N.) and 53.6 g. (S. N. D.) and on wheat diet containing whole wheat, pulse and vegetables 34.7 g. (G. C. N.) and 60 g. (S. N. D.). Out of thirty metabolism experiments the average protein requirement from ten experiments in which nitrogen balance was nearly obtained was found to be 46.4 g. protein per 70 kilo body-weight.

Protein nutrition on typical diets :—The experimental diets contained, on an average, about 80 g. protein and the addition of 10 oz. milk to these dietaries increased their protein contents by an extra amount of about 8 g. Metabolic data showed that the subjects were not only in systematic positive balance of nitrogen in all experiments but retained considerable amount of protein in their bodies. The average retention of protein on both vegetarian and non-vegetarian rice diets was about 13 g. and that on atta diets about 22 g. Addition of milk improved these retentions to 18 g. and 27 g. protein respectively. These results, therefore, would make it abundantly clear that the dietaries, as viewed from the standpoint of both protein contens and utilization, were adequate even without milk and hence could supply protein requirements. The addition of milk would only increase the margin of safety and provide first class protein, making the dietaries more satisfactory.

Biological value and digestibility of proteins in mixed diets :—The proteins of both the rice and wheat mixed diets are almost equally efficient, the mean biological value being 60 in both cases, but the rice diet is somewhat less digestible (80) than the latter (89.5).

Calcium.

Maintenance calcium requirement :—From an average of 36 experiments conducted on five different subjects with different diets at different periods, the maintenance calcium requirement was, as is evident from the following table (Table II), 0.432 g. per normal man per day.

Table II.

Experimental Subject.	Body weight kilo.	No. of Expts.	Calcium requirement per man-value.
B. C. R. S.	54	7	0.423
S. N. D.	49	7	0.407
G. C. N.	50	10	0.322
U. C. S.	47	7	0.426
S. N. D.	49	2	0.567
P. C. D.	45	3	0.451
Average.		36	0.432

Obviously, this value is remarkably similar to that of Sherman, namely, 0.45 g. per 70 kilo. body-weight. Accepting this value although not finally but only tentatively as the maintenance calcium requirement of an adult and adding a 50% allowance as is usual in nutritional practice, the calcium requirement for maintaining health activity is found to be 0.648 g. per man per day.

Calcium nutrition with typical dietaries and the effect of milk supplement :—The calcium contents of the rice-fish diets were found to be within the range, 193-280 mg., with a mean value of 236 mg., and that of vegetarian rice diet was 200 mg. whereas vegetarian atta diets contained about 300 mg. calcium in the average. If the calcium contents of these diets are compared with the human calcium requirement as discussed above, it will be clear that these dietaries are hopelessly deficient in calcium content in meeting even the bare maintenance requirement, not to speak of providing any extra allowance. This dark picture of calcium metabolism with Indian dietaries in general is not only the result of theoretical deduction but of direct metabolic studies.

In almost all experiments with rice-fish and rice diets, the subjects were in negative calcium balance and in the case of two subjects the total calcium outputs were such as to exceed the intake by about 192 mg. The addition of 10 oz. cow's milk increased the calcium intakes of these two subjects to 578 and 536 mg. respectively, and then one just maintained his balance while the other retained 69 mg. of calcium.

Similarly on vegetarian atta diets containing 302 mg. calcium, the experimental subjects lived in negative calcium balance, although the deficit was only small and not as prominent as with rice diets. With regard to calcium content and utilization therefore, wheat diets are better than rice diets, whether they be vegetarian or non-vegetarian. The addition of 10 oz. milk, increased the total calcium content of the wheat diet to 676 mg. and resulted simultaneously in a favourable retention of 269 mg. in a typical case.

The two most important points that evolve from these investigations are : (1) the Indian dietaries (without milk) are deficient in meeting the calcium requirements of the people and (2) it is necessary to incorporate some amount of milk to meet this deficiency. It may not be out of place to mention here that the amount of milk (10 oz.) used in the experiments covers the bare necessity and does not provide a safe margin.

Supply of calcium from chewing of betel leaves with betel-nuts, catechue and lime :—Although by far the richest source of calcium, milk and milk products are at the same time most costly. In view of the poverty of the large majority of the people a cheap source of calcium, of which an adequate amount is not present in ordinary diets, has to be found. Some experiments carried out showed that the chewing of betel leaves with lime and other ingredients as prevalant in many parts of the country supplied considerable amounts of calcium which were well utilised. This source of calcium is quite cheap and betel chewing is quite popular in India.

Phosphorus.

Maintenance phosphorus requirement :—The mean maintenance phosphorus requirement deduced from 13 experiments, which were carried out on different subjects with different diets and in which equilibrium was nearly attained, was 1.001 g. per day per adult weighing 70 kilo.—a value remarkably similar to the mean of the values of Sherman and of Owen,

Phosphorus nutrition with the diets :—The dietaries examined contained phosphorus which varied from 1334 to 1875 mg. with an average of 1623 mg. Comparing this with the phosphorus requirement it will be observed that the diets provided not only the maintenance phosphorus requirement but also more than covered the 50 per cent safety margin. When 10 oz. milk was added, dietary phosphorus increased to 1915 mg. which covered even 100 per cent margin. Actually all the subjects were in considerable positive phosphorus balance on all diets even without the milk and, on an average, retained 397 mg. and on the addition of milk there was a greater retention of phosphorus, namely 507 mg. So far as the metabolism of phosphorus is concerned, Indian dietaries are quite adequate and satisfactory.

Sulphur.

The different forms of sulphur, *viz.* total, inorganic, ethereal and neutral, in the urine of a subject on various diets containing adequate calories but different amounts of rice and wheat have been determined. It appeared that ethereal sulphates were purely products of detoxication of phenolic substances produced by the bacterial decomposition of dietary protein and hence the amount of ethereal sulphates excreted in urine might be assumed to be an index of intestinal bacterial putrefaction. Similar amounts of ethereal sulphates were found in the urine of the subject when fed on both rice and atta diets, while they were absent in the urine of the subject on protein free diet of adequate caloric content. It followed therefore that rice and atta diets were almost equally susceptible to bacterial putrefaction while non-protein diet was not.

Magnesium, iron and Manganese.

The results of some preliminary experiments regarding the metabolism of these elements showed that whole wheat diets supplied the bare minimum of magnesium required, while polished rice diets did not always contain this amount. Rice diets unless made up of highly polished rice contained the minimum requirement of iron and whole wheat diets contained a safe margin. As regards manganese, the experimental subjects were in positive balance on all diets.

19. Researches on the working efficiency of Indian labourers under Professor W. Burridge, Professor of Physiology, King George Medical College, Lucknow.

The present research has been carried out with Martin's Bicycle Ergometer. On this machine the subject pedals at varying speeds against a known resistance set up by a flexible band acting as a brake on a wheel of known diameter. The external work done by the subject is calculated from the tension of this band and the distance through which the rim of the wheel moves. The total heat production of the subject is found by collecting his expired air and analysing it. Next, from the tables of Zurtz relating oxygen consumption and respiratory quotient with caloric output,

the total energy expenditure of the subject can be calculated. The rates of these two energy outputs give the mechanical efficiency.

The first group of subjects was from the laboratory staff and after they had been tested we went outside for others. Unexpected difficulties were found, however, in respect of what may be termed the ignorant labourer. The apparatus frightened him and he would run away. Eventually, however, friendly co-operation with the Resident Engineer of the U. P. Electric Supply Coy. and the Manager of Dyer & Meakins' Brewery provided us with a regular supply of willing subjects.

At first we followed the methods employed in the classical experiments by Benedict and Cathcart, and like them and others we found that when work was hard respiratory quotients rose above unity. But beyond this unity, however, there are no data for calculating caloric output from the respiratory quotient and oxygen consumption. All that could be done at first, therefore, was to accept unity as the quotient for the basis of calculation.

Benedict and Cathcart believed that these very high respiratory quotients indicated that the supply of oxygen was inadequate. Their belief became reasonable after the existence of an oxygen debt from hard exercise was subsequently discovered. But it appears possible also that any raised respiratory quotient obtained during exercise is in itself evidence of an oxygen debt.

We are incidently examining this point because we have also found as have others that the respiratory quotient tends to rise with exercise. If this raised respiratory quotient be really evidence of the existence of an oxygen debt instead of a change in metabolism as hitherto presumed, calculations of mechanical efficiency on the latter basis are invalid. So far our calculations have been made on this latter because such was customary, and none has yet taken this debt into account when estimating efficiency.

With those reservations, it may be noted that we tend to find the mechanical efficiency of Indian labourers to be slightly higher than those found by Benedict and Cathcart in America. They found an average value of 21% where we are finding 22%. Here and there, however, we find individuals of consistently low efficiency. We can also confirm the findings of others that mechanical efficiency is lessened at the higher speeds. If, however, the oxygen debt be taken into consideration, our figures for efficiency at high speeds will certainly be found different from those which we have hitherto calculated.

It may be appreciated from the above that, after making a large number of experiments and subsequently calculating mechanical efficiency along traditional lines, we have become aware that the oxygen debt disturbs our results. Our immediately pressing problem is, therefore, to ascertain how much disturbance that debt makes. That is what we are now attempting in particular.

We further observed that the resting respiratory rates of our subjects were higher than we first believed they ought to be, our belief being based on the figures given in text-books published in Europe and America. Since these seeming high rates could indicate emotional disturbance and so be a disturbing factor in the work done, the resting respiratory rates of all patients with normal temperatures in King George's Hospital were examined over several days. From these observations the normal respiration rate when lying down in India was found to be 20 per minute,

20. Enquiry into malnutrition amongst Bengali boys with regard to vitamin B_1 and C under Dr. N. M. Basu at the Presidency College, Calcutta.

Vitamin C enquiry.

The following investigations were undertaken :—

Assessment of the state of saturation of Bengalee boys with regard to vitamin C :—The results obtained are :—

(*a*) about 60% of the boys examined were unsaturated, most of them requiring 2,000 mg. of vitamin C to be completely saturated.

(*b*) the urinary excretion of vitamin C is not an indication of the state of saturation unless it exceeds 30 mg. per day.

(*c*) certain persons, who had no manifestations of scurvy, were excreting such low amounts of vitamin C as were noticed only in scurvy patients in other countries.

The optimum requirements of vitamin C by the average Bengalee :—The experiments were carried out as follows :—Certain persons were saturated with vitamin C by the daily ingestion for some time of heavy doses of ascorbic acid. Their intake was then reduced by stages to such an amount as caused a constant excess excretion of 30% of the excess intake. This amount was taken to be the optimum requirement of the subject under investigation.

It was found that the optimum requirement of the persons examined was about 70 mg. and was not appreciably influenced by their different dietetic habits.

The course of excretion of ascorbic acid :—The results obtained are :—

(*a*) after an intake of the vitamin in heavy doses the excess output following the intake is much higher in the first period of 12 hours than in the subsequent period, the difference in the output between these two periods increasing with the total excess output in 24 hours, *i.e.*, with a higher state of saturation of the body.

(*b*) the excretion of the vitamin rarely becomes constant even when the body is completely saturated and is always less than the amount ingested, unless some unknown factors cause depletion of the stores of the vitamin in the body and thereby raise the percentage of excretion beyond 100.

The effects of vitamin C on the strength of contractions of plain, cardiac and voluntary muscles of animals and the incidence of fatigue in skeletal muscles (i.e. voluntary muscles) :—The results obtained are :—

(*a*) vitamin C definitely augments the contractions of all these muscles, the augmentation being independent of changes in pH that may occur in Ringer's solution on the addition of the ascorbic acid.

(*b*) it markedly delays the onset of fatigue in skeletal muscles.

The effect of vitamin C on the onset of fatigue in finger muscles of human beings :—It has been shown that ascorbic acid augments the contractions of these muscles and markedly delays the onset of fatigue.

The influence of vitamin C on the coagulation-time of the blood of guinea-pigs :—When guinea-pigs are made scorbutic, the coagulation-time of their

blood increases considerably. With the administration of vitamin C, it begins to decrease and becomes normal after about 3 weeks, if vitamin C intake is continued.

VITAMIN B_1 ENQUIRY.

Assessment of the state of saturation of Bengalee boys with regard to vitamin B1 :—Eight persons have been examined by the long but reliable rat-growth method of biological assay. The normal excretion of B_1 in urine of these persons and the excretion after the ingestion of one or more test doses of vitamin B_1 have been ascertained. The diets of these persons, especially the carbohydrate content and the total calorie intake, have been recorded. It has been shown that :—

(*a*) the average normal excretion of thiamin varied between 6.5 to 28 I. U. According to Harris & Leong figures for average excretion range between 30 to 90 Y., *i.e.*, 10 to 30 I. U.

(*b*) following the method of Harris, it was found that, of these 8 persons, three were definitely unsaturated, four were on the border-line, and one only was saturated.

Six persons have been examined by the recent chemical method of estimation of vitamin B_1 as modified by Wang and Harris. As in this method the fluorescence of the test samples of urine is compared by direct vision with the eye with that of a standard solution of vitamin B_1, errors are bound to creep in. Modifications have, therefore, been introduced, limiting the error within a small range. These modifications will be described in detail in the paper to be published shortly.

Out of six persons examined, two were found to be normal, one deficient and three others were on the border line. None of these subjects showed any clinical symptoms of vitamin B_1 deficiency. Further work is in progress to ascertain whether the standards laid down by Harris for the assessment of vitamin B_1 deficiency are applicable in this country.

21. Enquiry into malnutrition among Bengalee boys with regard to vitamin A deficiency, under Dr. N. M. Basu at the Presidency College, Calcutta.

ASSESSMENT OF MALNUTRITION OF BENGALEES WITH REGARD TO VITAMIN A BY JEANS' BIO-PHOTOMETER.

This was done in accordance with the directions of the author of the instrument, and the dial readings of the persons examined were correlated with their dietary intake. The results were :—

(*a*) the average dial reading of persons taking an adequate amount of vitamin A was 25 with the probable minimum of 16.

(*b*) of the 40 persons examined, eleven had very low dial readings indicating that they were unsaturated and another was a border-line case.

(*c*) there were nine cases which showed discrepancy between dial readings and dietary intake of vitamin A.

Although the instrument gives a rough index of the vitamin A status of the body, it needed improvement along the lines of Hecht.

ASSESSMENT OF VITAMIN A DEFICIENCY WITH THE SAME INSTRUMENT AFTER MODIFICATION FOR OBSERVING BOTH PERIPHERAL AND CENTRAL ADAPTATION.

Hecht's technique of assessment was followed. Fifty-four persons have been already examined. The results were :—

(*i*) a close correspondence is obtained between the dietary intake of vitamin A and the minimum adaptive values of light in the periphery of the retina, as indicated by the dial readings of the instrument. Thus a person taking daily about 500 I. U. of vitamin A showed a dial reading (D. R.) of 56. After taking in addition one cup of milk and two eggs daily for seven days, he showed a D. R. of 66. A person consuming over 2,000 I. U. of vitamin A daily gave a D. R. of 70, and another with a daily intake of over 4,000 I. U. of vitamin A showed a D. R. of 88.

(*ii*) according to Hecht, the minimum peripheral adaptive value in a normal person, for violet light, is such as would correspond to a D. R. of 80. As white light whose adaptive value is somewhat less than that of violet light, is used in these experiments, the minimum peripheral adaptive value in a normal person may be taken to correspond to a D. R. of about 70 instead of 80. The dial readings with white light of persons of different ages and in a condition of saturation with vitamin A are being investigated in this laboratory. Until this investigation is over, the D. R. of 70 is taken provisionally to be the normal standard for Bengalees. Of the 54 persons examined 30 were college students (age 17 to 24) and 24 were employees of moderate income (age 18 to 50). Twelve students and 11 employees had D. R. below 70, or, in other words, the percentages of vitamin A deficient persons in the two groups, were 40 and 46 respectively.

A RAPID METHOD OF ASSESSMENT OF THE VITAMIN A STATUS OF PERSONS.

In Hecht's method the person examined is required to have his eyes exposed to a bright light for a period of 3 minutes which is considered sufficient to bleach completely the visual purple of his retina. The restoration of his vision in the dark, which corresponds to regeneration of his visual purple, is then examined by a momentary flash of a light (coloured) of gradually diminishing intensity. The minimum intensity of light that is appreciated with prolonged rest of his eyes in darkness, is considered to be proportional to the availability of vitamin A in the retina for regeneration of visual purple and therefore, to the vitamin A content of his body. It appears from Hecht's figures that all persons whether normal or deficient in respect of vitamin A attain their maximum rod-thresholds within 20 to 30 minutes. Dial reading remained practically constant on further sitting in the dark, and in fact, the maximum dark adaptive value of an individual has been taken as a criterion of the vitamin A status of

his body. It is evident from the above that one of the important criteria of the vitamin A status of a person is the minimum intensity of light that is appreciated on prolonged rest of his eyes in darkness. It has therefore been thought desirable to dispense with the preliminary exposure of one's eyes to bright light and to take readings with the instrument for examining his dark adaptation till he reaches the state of maximum dark adaptation. An advantage of this method is economy of time. A batch of six to twelve persons can be taken at once into a dark room and can be examined one after the other by exposing their eyes to momentary flashes of light of low intensity till each of them arrives at the state of maximum dark adaptation. The lowest value of light that is appreciated by an individual is noted. In this way twelve persons can be easily examined within one hour, whereas according to Hecht's method the examination of a single individual requires nearly 40 minutes. Thus the modified method, although based on the principles of Hecht's method, is much quicker and is expected to yield equally accurate results.

Forty-five persons have been examined by this modified method. Of these 29 were well-fed school students (age 13 to 16) and 16 were adults (age 20 to 50). The results are shown below :—

(*i*) taking the D. R. 70 as the provisional standard for normals, it was found that out of the 29 school boys only two (*i.e.* about 7%) were deficient while of the 16 adults 9 persons (*i.e.* 56%) were deficient in vitamin A.

(*ii*) three deficient cases (with D. R. below 60) and 3 normal persons (with D. R. of 80) were given daily for 8 days 12,000 I. U. of vitamin A free from vitamin D. The former three persons showed considerable improvement while the readings of the latter were but slightly changed.

Further work is in progress to examine this method in all its bearings. If the method is found to yield satisfactory results, it would be extremely useful for a rapid assessment of the vitamin A status of a large number of people within a comparatively short time.

Determination of the absorption of carotene by rat test, from different leafy vegetables after cooking in the usual way prevalent in Bengal.

As foods containing vitamin A are rather costly and not available to the poor, it was considered necessary to determine the availability of carotene from cheap leafy vegetables (cooked) so that the deficiency of vitamin A may be made good by their intake. The work is in progress. So far, three leafy vegetables most commonly used in Bengal, have been examined, and it was found that, at the same level of intake of carotene (*viz.* 20 I. U.) from these sources, about 60% was absorbed in each case.

The inter-relationship between carotene, vitamin A, total acidity, pH and sugar content of different varieties of mangoes during the process of ripening.

This was determined jointly by the workers attached to two different enquiries. Twenty mangoes of seven varieties were examined.

(*a*) the diminution of vitamin C is accompanied by an increase in carotene,

(*b*) the diminution of vitamin C occurs *pari passu* with the diminution of the acidity of the fruit while ripening takes place.

(*c*) the increase of carotene on ripening is accompanied by an increase of the sugar content of the fruit.

(*d*) the variations in pH are more or less parallel to the variations in the acidity of the fruit.

Further work is in progress.

22. Enquiry into the detection of food adulteration under the Public Analyst, Assam, at the Public Health Laboratory, Shillong.

Some of the methods of analysis at present practised for the detection of adulteration of foods-tuffs as well as some of the standards laid down under the Pure Food Acts have been found insufficient and cause considerable difficulty in detecting skilfully adulterated samples. This enquiry has been undertaken with a view to developing better methods of analysis as well as supplementary standards, wherever necessary, for Indian foodstuffs. In short the object of this enquiry is to help towards the efficient working of Pure Food Acts.

The first problem taken up by this enquiry has been the detection of skilfully adulterated buffalo milk, which can be easily passed as cow milk, by keeping the standards (specific gravity, fat and solids-not-fat) within the legal limit for cow milk. During the period under consideration, 755 samples of genuine milk from known breeds of cow and buffalo (as advised by Sir Bryce Burt in a letter forwarded to this enquiry by the Secretary, Indian Research Fund Association) were analysed. The majority of these milk samples were obtained from Government cattle farms, the rest were obtained from animals milked in the presence of health officers and sanitary inspectors. As far as possible, the samples were procured from different places in the province. Of these 755 samples, 514 were cow milk and 241 were buffalo milk. On analysis it has been ascertained that in genuine buffalo milk the specific gravity may be as high as 1040, fat 11.2 per cent and solids-not-fat 15.6 per cent. A fat content of 8 to 9 per cent and solids-not-fat 12 to 13 per cent are quite common in the buffalo milk of this province. Dilution of buffalo milk with water or starch emulsion (in practice the dishonest vendor often uses the *mari* or supernatant water from boiled rice) may be carried out in such a way that the specific gravity, fat and solids-not-fat remain within the standards 1028, 3.5 and 8.5 legally prescribed for genuine cow milk. During the progress of this enquiry such mixtures were successfully prepared in the laboratory. The analysis of genuine samples of milk revealed that in comparison with other constituents (fat, solids-not-fat etc.,) the lactose contents of cow and buffalo milk vary within a very narrow range and that cow milk contains 4 to 5.15 per cent lactose and buffalo milk 4.2 to 5.25 per cent, the average value being 4.75 per cent for both. Dilution of buffalo milk, if it is to be made profitable, brings the lactose value below 4 per cent. which is the minimum suggested by this enquiry for cow and buffalo milk. The work carried out by this

enquiry has proved that the estimation of lactose will facilitate the detection of adulterated buffalo milk which is passed as cow milk.

For rapid estimation of lactose in milk, a volumetric method in which acetic acid and dialysed iron are used for quickly preparing a clear filtrate suitable for Fehling titration with methylene blue as internal indicator, has been worked out during the current year. While standardising this rapid method, parallel colorimetric and polarimetric estimations of lactose were also carried out and comparative values of all these methods were assessed. The rapid method worked out by this enquiry has got a distinct advantage over the old method in which alumina cream was used for obtaining a clear milk filtrate and acidified potassium ferrocyanide was used as outside indicator during Fehling titration.

Work on the determination of freezing point of milk was also taken up during the current year. Fresh samples of cow milk showed a freezing point varying from—0.545° C to—.550° C. It has been found that the addition of formalin causes an anomalous shifting of the freezing point (—0.580° C to—.640° C.) and hence its determination is of no use in the routine examination of milk samples which as received in this laboratory are mostly formalised.

The second problem which has been taken up by this enquiry is the detection of adulterated buffalo ghee which is passed as cow ghee (keeping the standards, Reichert value, saponification value etc. within the limit demanded for cow ghee by the Assam Pure Food Act, 1932.) It has been observed that buffalo ghee (minimum Reichert value 30) adulterated with 15 to 20 per cent of vegetable products (hydrogenated oils), satisfy the standards (Reichert value 24, saponification value 222 and butyro-refractometer reading 40-42.5 at 40° C) for cow ghee. Analytical work on samples of genuine buffalo ghee, cow ghee and vegetable ghee, in order to determine the butyric acid value as well as the limits within which the standards Reichert value, saponification value etc. vary, has been started. It is proposed to continue this work and after analysing buffalo ghee adulterated with known quantities of vegetable or animal products, to devise easy methods for the detection of such adulteration.

23. Bengal fish enquiry under Prof. B. C. Guha at the University College Science and Technology, Calcutta.

PROTEIN, FAT AND MINERAL CONTENT OF FISH.

Analysis of the edible portions of a large variety of Bengal fish regarding their water, protein, fat, calcium, phosphorus, total and ionisable iron content has been carried out. Among 37 species so far analysed, *hilsa* (*Clupea ilisha*) and *dhain* (*Silonia silundia*) are the richest sources of fat. *Shinghi* (*Saccobranchus fossilis*) and *Pabda are* the richest in protein. *Parsey* (*Mugil parsia*), *Rohu* (*Labeo rohita*), *Shinghi* and *Katla* (*Catla catla*) have got the largest content of calcium. *Parsey* (*Mugil parsia*) is the richest source of phosphorus. *Magur* (*Clarius batrachus*) and *Koi* (*Anabas testudineus*) gave the highest figures for ionisable iron.

As certain varieties of fish are not available throughout the year, it is necessary to carry on the investigation from season to season. The seasonal variations in the nutritive value of the same species of fish are also under study and the work is being extended to include the vitamin values of fish.

Available iron in fish tissue.

Only the ionizable iron content of food-stuffs has been so long considered to be nutritionally available and this has been estimated by the αα′-dipyridyl method of Hill. On a critical examination of the results obtained by the dipyridyl method, compared with those obtained by biological assay, it appeared that there is some iron not determined by the dipyridyl, which is nevertheless, nutritionally, available. This cannot be entirely accounted for as haemoglobin and haematin and the nature of this iron fraction has not yet been ascertained. The action of proteolytic enzymes (pepsin and trypsin) on fish tissue has been investigated in order to throw light on the question. From the results it appears that some of the iron present in fish tissue is present as an iron-protein complex, which, as such, does not react with αα′-dipyridyl, but which is readily hydrolysed by pepsin and trypsin and is, therefore, nutritionally available. After digestion, the iron liberated from the iron-protein complex can be estimated by the dipyridyl method of Hill. A method of estimation of the total available iron has been developed which involves peptic digestion followed by reduction with sodium hydro-sulphite and treatment with dipyridyl. The available iron estimated by this technique is much greater than that obtained with the undigested tissue by Hill's method. This method is also applicable to other animal tissues. Some other difficulties, which are met with in Hill's method, do not arise in this new method. During the formation of roe, the available iron content of the muscle tissue of the fish is greatly decreased while iron is found concentrated in the roe. The nature of the iron-protein complex is under further investigation.

Biological value of fish proteins.

The biological values of three different varieties of Bengal fish, which are commonly consumed, namely *chingri*, *Shinghi* (*Saccobranchus fossilis*), *Air* (*Arius arius*) have been investigated by the rat-growth method. From the findings it appears that the biological values of *Chingri*, *Shinghi* and *Air* at 5 and 10% levels are superior to that of casein. Compared among themselves, *Air* is superior to both *Chingri* and *Shinghi* at 5% level, while at 10% levels they are all approximately equivalent. Further investigations with other varieties of fish are in progress.

Effect of cooking on the digestibility of fish proteins.

The effect of different methods of cooking—frying and boiling for different periods of time—on the digestibility of the proteins of fish have been studied. The digestions have been carried out *in vitro* by pepsin, trypsin and by pepsin followed by trypsin. From the results, it appears that the rate of digestion varies from species to species, which is apparently connected with the differences in the composition of the protein in fish. The rate of digestion of fish under-cooked in boiling water is greater than that of raw, fried or thoroughly cooked fish. This may be due to possible destruction of the anti-enzymic properties in the under-cooked fish, while the proteins at this stage are not markedly denatured, which might have

hindered the rate of hydrolysis. With all the species under investigation, the rate of hydrolysis is found to be greater in peptic digestion than in tryptic, except in two cases *viz* : *Boal* (*Wallagu attu*) and *Shole* (*Opheephalus straitus*), where the results are reversed. When the peptic digestion is followed by tryptic digestion, hydrolysis is more complete. In general, the digestibility is the greatest with under-done fish as compared with raw, fully cooked or fried fish. This work is being continued with other varieties of fish.

24. Enquiry into calcium metabolism in health and disease under Dr. V. N. Patvardhan at the Seth G. S. Medical College, Bombay.

During the year the investigations on (1) the absorption of calcium from the intestine (2) the excretion of calcium into the intestine (3) influence of vitamin D on the composition of bone and (4) the calcium and phosphorus contents of soft tissues were continued.

The absorption of calcium.

(*a*) *The route of absorption* :—Solutions of calcium salt, lactate or gluconate, were introduced into the duodenum of anaesthetized dogs and the concentrations of calcium in the portal blood and mesenteric lymph were estimated. The results showed that, during the absorption of calcium, the concentration of the latter increased both in lymph and in portal blood. The amount and the course of rise were practically the same in both the fluids showing thereby that calcium, after being absorbed from the intestine, enters the general circulation by the portal as well as by the lymphatic route. No relationship between the dose of calcium and its maxium rise in blood during absorption could be established.

(*b*) *The influence of vitamin D on the absorption of calcium* :—In order to study the influence of vitamin D on the absorption of calcium, Verzar's technique of the isolated loops *in situ* was employed, details of which have been given in last year's report. The experiments were carried out on (1) young rats on rachitogenic diet (2) young rats on rachitogenic diet supplemented with 60 I. U. of vitamin D per rat per day (3) young rats on stock diet (4) adult rats on stock diet (5) adult rats on stock diet receiving massive doses of vitamin D (6) young rats receiving diet deficient in vitamin D and (7) young rats receiving vitamin D deficient diet supplemented with 60 I. U. of vitamin D per rat per day. A statistical analysis of the results obtained showed that vitamin D did not influence either the rate or the magnitude of absorption of calcium from the intestine.

The excretion of calcium.

The influence of vitamin D on the excretion of calcium was studied by balance experiments performed on rats receiving calcium defcient diet and heavy doses of vitamin D by subcutaneous injections. The results showed that vitamin D in heavy doses caused an increased excretion of calcium into the gut even in a condition of calcium starvation. It is

suggested, therefore, that vitamin D might probably be a controlling factor in the excretion of calcium by the intestinal route.

THE INFLUENCE OF VITAMIN D ON THE MINERAL COMPOSITION OF BONE.

The effect of vitamin D on the mineral composition of bone was studied by analysing the bones of albino rats for their ash, Ca, P and CO_2 contents. The bones were obtained from groups of rats 1 to 5 mentioned above.

(*a*) *Ash* :—The results of analyses have shown that the mean ash content of bones of ricketic rats was the least, while that of the corresponding controls on the stock diet was the highest. The ash content of bones of hypervitaminotic rats in one series of experiments was reduced while in the other series of experiments, where the Ca and P content of the diet was much higher, it remained unaltered. It showed that the demineralisation of bone in hypervitaminosis D probably depends upon the Ca and P content of the diet.

(*b*) *Ca, P and CO_2 content of bone* :—The ratio of $\frac{total\ Ca}{P}$ in the bone ash was practically the same in all the groups. The main variable feature was the CO_2 content of bone. It was higher in the ricketic group than in the corresponding control on the stock diet. The carbonate content of bone appeared to increase with age. In the group receiving excessive doses of vitamin D, Ca and CO_2 content of bone appeared to be reduced. These findings, considered together with the observations on the absorption of calcium, suggested that vitamin D is primarily concerned neither with the absorption of calcium from the intestine nor with its deposition in the bone but with the control of some other function or functions in which calcium is intimately involved.

THE CALCIUM AND PHOSPHORUS CONTENTS OF SOFT TISSUES.

The analyses of soft tissues—(brain, muscle, spleen, testes, liver, heart, lungs and kidneys) of albino rats for their Ca and P content have shown that the Ca content of brain decreased and that of lung increased in rickets while its quantity in other tissues remained practically unaltered. In hypervitaminosis D the Ca contents of almost all soft tissues, so far examined, increased without any sign of pathological calssification. The data regarding the P content were more variable and hence less liable for a clear interpretation.

25. Tissue culture enquiry under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta.

The enquiry started on the 1st of April 1939, with a staff consisting of an Experimental Cytologist and a technician. The latter post could not be filled up for the first five months for want of a suitable candidate and the Experimental Cytologist being new to the work, no great progress

could be made during the first quarter and the greater part of the second. During this period the Experimental Cytologist underwent a training in the theoretical and practical aspects of tissue culture in its various branches including histology and microphotography and also did considerable library work. From September onwards, the enquiry being fully staffed and the Experimental Cytologist having picked up the technique, a fair amount of work has been put in.

Successful cultures in vitro of connective tissue and epithelial cells from the embryo chick have been obtained in hanging drop preparations, which is a very suitable method for microscopical study. The cultures were further studied after fixing and staining them with different dyes at various stages of growth. The factors governing active growth are being studied, before applying the technique to the projected study of cell nutrition. Microphotographs of the various stages of growth as well as the appearances of the mitotic cycle are being taken. Recently, experiments with duck embryos were undertaken as fertile chick eggs are difficult to obtain during certain seasons of the year. Successful cultures of the heart and liver of the embryo duck have been obtained and are being studied further. Subcultures of growing cells with fresh nutriments have been undertaken for lengthening the life and duration of active growth and have been fairly successful as evidenced by comparisons with controls, where the growing cell has the tendency of rounding up with cessation of growth after a certain period, whereas in the subcultures growth and multiplication was prolonged.

26. Enquiry into dental caries under Dr. M. K. Rahman Siddiqi at Cawnpore and Shahjahanpur.

The problem of eradication of dental caries is of great importance to the health of any community as dental caries directly or indirectly causes much suffering. It is known from Dr. May Mellanby's pioneer work (1918-1934) that certain factors in diet and especially vitamin D, the calcifying vitamin, play an important part in the normal developement of teeth. She has also shown that the better the structure, other conditions being the same, the less the liability of teeth to decay. She has arrived at this conclusion by methods of estimating the quality of the structure in human teeth. These methods have been worked out by her with as scientific a precision as possible in practice; all grades varying from normal or perfect to varying degrees of hypoplastic structure are found. According to these criteria, the majority of the teeth of British children are hypoplastic and it was thought that if the same criteria were used in examining the teeth of children in India, a higher percentage of defects might be discernible than has hitherto been believed.

The problem thus appeared very fascinating for investigating the association of these defects with dental caries in India and working with the same criteria and methods for finding the effects of different diets among various communities in this country.

The work on the above problem under the present enquiry could be started on May 30, 1939 only during the spare hours of the writer, when free from routine official duties. The plan of work has been to survey the individual teeth of children between the ages of five and twelve years and record the presence of varying grades of hypoplasia and caries in deciduous and permanent teeth on a "Dental caries investigation and survey card". In addition the following points have also been noted on the card :—

1. age. 2. area (urban or rural) 3. name 4. community-Muslim (Syed etc.), Hindu (Brahmin, Kayastha etc.), Christian (converted etc.) 5. province 6. physical measurements—height, weight, arm girth, chest depth, hip-width, etc. 7. any bony deformities 8. diet (number of meals and the details of food taken) 9. social status 10. hygienic condition of home and its environment 11. amount of clothing worn and exposure to sunshine 12. family history 13. history of illnesses 14. present state of health 15. nutrition 16. systemic diseases, if any 17. anaemia and 18. any other information.

The methods of grading the extent of dental caries and hypoplasia, observed in the teeth examined, have been adopted from those initiated and developed by Dr. May Mellanby in the course of her researches on teeth. The following points have been carefully noted :—

(*i*) the condition of gums, healthy, inflamed or spongy.

(*ii*) alignment or occlusion by suitable symbols.

(*iii*) the presence and state of eruption of teeth.

(*iv*) the structure of fully erupted teeth as evidenced by surface enamel—divided into five grades :—Normal structure (N), slightly defective (H1), defective (H2), severely defective (H3), and grossly defective (H4).

(*v*) the presence, situation, size and texture of carious areas, dividing them into three grades—slight caries (C1), medium caries (C2), and advanced caries (C3).

In this way a record of teeth and other particulars for about 450 children has been carefully prepared up to the end of November 1939. In addition ground sections of some extracted teeth have also been prepared with a view to noting the proportional thickness of enamel and dentine.

Analysis of the data obtained.

A considerable amount of time is being devoted to the examination of the data collected. The information is being arranged and charted under different heads for deciduous and permanent teeth separately, in order to find out to what extent any one group of children differs from the other in respect of the incidence of dental caries and hypoplasia and thus possibly be able to detect any group which may relatively be immune to these conditions and to find another group in which the incidence may be comparitively very high. With this object in view the charting is done under the following heads separately for deciduous and permanent teeth :—

1. incidence among Muslims, Brahmins, Kshatryas etc. and scheduled castes.
2. incidence in urban and rural areas.

3. incidence among children of different nutritional status.
4. incidence among children with different dietary intake, (a) vegetarian and non-vegetarian. (b) showing different dietary deficiencies.
5. incidence among children of different age groups between five and twelve years.
6. incidence among children of different social status.

TENTATIVE CONCLUSIONS.

No definite conclusions can yet be drawn until several thousands of children have been surveyed in different areas. The following observations, however, seem apparent and instructive :—

1. contrary to what is popularly held, the incidence of caries appears somewhat higher in rural than in urban areas though it is not possible to state this definitely as the children examined in the rural area have been mostly Hindus ;
2. incidence among different age groups seems to indicate that the lower the age group, the higher the incidence of caries ; this is because incidence of caries is much higher in deciduous than in permanent teeth ;
3. so far vegetarians have shown a higher incidence of caries than non-vegetarians ;
4. nutritional status has so far shown no obvious relation with the incidence of these conditions ;
5. among the different communities so far examined, the incidence of caries and hypoplasia appears definitely less in Muslim children than in the various castes among Hindus, the average hypoplasia and caries figures for the latter being almost the same as for British children, as reported by Dr. May Mellanby. The following figures illustrate this :—

Average hypoplasia figure

Muslims—0.55 ; Brahmins—0.94 ; Scheduled Castes—1.09 ; * English—1.04.

Average caries figure

Muslims—0.20 ; Brahmins—0.31 ; Scheduled Castes —0.30 ; * English—0.395.

* (Pages 36 and 37 of the final report on " The influence of diet on caries in children's teeth " by Dr. May Mellanby).

LEPROSY.

27. Leprosy enquiry under Dr. J. Lowe at the School of Tropical Medicine, Calcutta.

CLINICAL WORK.

(*i*) The study of seasonal variations in the clinical and bacteriological findings in leprosy has been continued.

(*ii*) Surgical removal of the sheath of the ulnar nerve has been performed in a number of leprosy patients suffering from acute neuritis. The procedure has been found to be of great help in relieving the pain and it appears to be effective in the prevention or minimisation of deformity.

(*iii*) Total excision of the skin lesions has been performed in a number of early cases of the neuro-macular type of leprosy and the patients have been watched for signs of relapse. The period of observation in the majority of cases is too short to allow any definite conclusions to be drawn but the results suggest that if cases are suitably selected the complete excision of a lesion is not likely to be followed by a relapse, at least in a certain percentage of cases.

COMPLEMENT FIXATION IN LEPROSY.

A study has been made of the complement fixation test in leprosy using antigens from six different acid-fast bacteria. These antigens were prepared from cultures of the so-called lepra bacilli of Kedrowsky, Llera, Bayon and Duval according to the W. K. K. method. An antigen prepared from Mycobacterium phlei and the W. K. K. antigen from tubercle bacillus were also included. The tests were done in 123 cases of leprosy and in 60 cases of other diseases. The study was undertaken to find out if the test would give any evidence regarding the etiological relationship of these organisms to leprosy. All the antigens behaved more or less similarly, though the one prepared from Llera's organism gave a higher number of positive results than the other antigens. The greater sensitivity of Llera's antigen, however, does not seem to depend on any specificity as it was observed in non-leprosy cases also.

BACTERIOLOGY.

(*i*) Attempts to cultivate the organisms of rat leprosy and human leprosy have been continued. No definite multiplication has been observed.

(*ii*) It was reported last year that an acid-fast bacillus resembling rat leprosy bacillus had been isolated from rat leprosy material by tissue culture methods. When inoculated into rats it did not, however, produce generalised infection in these animals. It was, therefore, considered that it was not the causative organism of rat leprosy. The organism in question has been studied further and it is interesting to note that in the second passage in rats it has produced generalised infection in these animals with lesions similar to those seen in experimental rat leprosy. The organism is being studied further.

(*iii*) The action of methylene blue on the rat leprosy bacillus has been studied in vitro and in vivo.

(*iv*) The action of sulphanilamide and M. B. 693 on the organism of rat leprosy is being studied with a view to finding out if the drugs have any bactericidal effect on this organism.

ANIMAL EXPERIMENTS.

(*i*) *Syrian hamsters.* A number of hamsters, some splenectomised and some not splenectomised, were inoculated with human leprosy material. Half the animals were inoculated intraperitonially with an emulsion rich in leprosy bacilli and in the other half a small piece of leprosy nodule was put under the skin of each. Two of the animals died within 8 months after inoculation, 12 were sacrificed 9½ months after and 3 an year after inoculation. In none of the animals was any macroscopic or microscopic evidence of leprotic infection found. Adler's work regarding the susceptibility of hamsters to human leprosy has thus not been confirmed.

(*ii*) *Splenectomised monkeys.* Six monkeys were splenectomised and after the splenectomy wound had healed were injected intraperitonially with emulsions rich in leprosy bacilli. Four of the monkeys died within 5 months of inoculation. In one of the animals nothing abnormal was seen at post-mortem examination and microscopically only a few acid-fast bacilli were found in omentum and mediastinal glands. In the other three the appearances were suggestive of a massive tubercular infection. The matter is being investigated further.

BIOCHEMICAL WORK.

(*i*) Lecithin contents of whole blood and of plasma were estimated in 28 cases of leprosy. The values obtained appear to fall within the normal range.

(*ii*) Blood lipase has been determined in 95 neural and 42 lepromatous cases of leprosy and in 14 non-leprosy cases. In the lepromatous cases the average amount of butyric esterase in terms of $\frac{N}{50}$ NaOH was 0.81 in half a c.c. of serum, in the neural cases the average was 1.11 while in the non-leprosy cases it was 1.05.

28. Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leper Settlement and its associated Clinics.

The leprosy enquiry undertaken during the year 1939 was enlarged and covers various aspects and this report is summarised under the following heads: (*a*) dietetic experiments; (*b*) therapeutic investigation; (*c*) clinical investigation; (*d*) field investigation; (*e*) animal experiments.

Dietetic experiments.

(1) *Skimmed milk.* The administration of skimmed milk in children and wheat diet in adults has been continued. The children selected for this experiment have been divided into those under observation and those under treatment. The former group is a smaller group and chosen from material available at the Silver Jubilee Children's Clinic, Saidapet. There are 35 children in this group with a similar number of controls and the findings of last year were confirmed that the administration of skimmed milk seems to have no influence in assisting the milder lesions to subside more rapidly. The children under treatment were selected from the Silver Jubilee Children's Clinic and the Lady Willingdon Leper Settlement and are a larger group. The following is a comparison of the results over a period of a year and six months respectively :—

Table I.

January 1937—*December* 1938.

		Negative	Percentage
Skimmed milk	33	19	57%
Control	70	15	20%

Table II.

January 1939—*July* 1939.

		Negative	Percentage
Skimmed milk	36	8	27%
Control	47	5	10.6%

It will be noted that the percentage of *negatives in the second group (*i.e.* in the group January 1939—July 1939) dropped from 57%—27%, but the period is just half and further in the controls the negative group has also dropped from 20%—10.6%. If the two statements are consolidated the following are the findings :

Table III.

		Negative	Percentage
Skimmed milk	69	27	39.1%
Control	117	20	17.0%

It is seen that the negatives in the skimmed milk group still remain more than twice as high as in the control group. We believe that the factor of unconscious selection cannot be completely ruled out in the first group of cases, and therefore a completely fresh group has been chosen as from January 1939. The method adopted in choosing the cases, was placing on admission every alternate lepromatous child on skimmed milk. Thus the factor of unconscious selection was eliminated. We have now a group of 61 cases with an adequate number of controls. If at the end of another year these findings are confirmed we feel that then we shall be justified in drawing conclusions.

* A negative case is one in which the Mycobacteria leprae have disappeared from the skin and nasal mucosa on examination by standard methods.

(2) *Wheat Diet* :

Nerve pain as a sequela in leprosy is a symptom which causes considerable distress and many remedies have been suggested. Operation or injection of alcohol is feasible when only one or two nerves are involved, but these are not practical procedures when the nerve pain is general and of a persistant gnawing nature. It was suggested that wheat diet might be of value in cases of severe and chronic nerve and bone pain. The latter is not a sequela of leprosy, but a common complication ; the bone pain is frequently so severe that the slightest pressure gives rise to the most excruciating pain.

Thirty-seven cases in all have been observed. In every case in which the wheat diet was consumed for three months and more, there has been either complete relief of these distressing symptoms or they have been very considerably alleviated.

Table III.

Percentage of cases showing complete or *partial relief of nerve and bone pain after the administration of the wheat diet.

	Complete relief		Partial relief		Total	Percentage	
	M.	F.	M.	F.		Com. Rel.	Par. Rel.
(a) Bone pain	7	5	0	2	14	85.7%	14.3%
(b) Nerve pain	12	4	6	1	23	69.5%	30.5%

Total No. of cases 37. Total completely relieved 75.7%

All rice was eliminated from the diet and instead whole wheat was taken. This was lightly ground on the curry stone and cooked in the same way as rice and distributed to the patients who ate it along with their usual curries. In the evening chappatties were eaten. For adults 21 ozs. of wheat per diem were supplied and for boys 12 ozs.

Table IV.

Analysis of cases indicating the condition as regards leprosy after the administration of wheat.

Negative	Much improved	Improved	Stationery	Worse	Total
2	2	18	14	1	37
5.1%	—	(9 F.)	(3 F.)	—	—

This experiment was pursued for one year and nine months and the relief of nerve and bone pain was so consistent and frequently so striking that we feel justified in concluding that the administration of wheat diet is a powerful therapeutic measure in the treatment of chronic nerve pain in leprsoy, and in the relief of the severe bone pain which is not directly due to leprosy but a common condition in South India, the etiology of which we believe is not clearly understood.

* *Partial :* Slight tenderness still present and occasional pain but not severe.

Therapeutic investigation.

This has been pursued during the year along the following lines :—

i. trial of new remedies.

ii. investigation of the optimum dose of the hydnocarpus preparations.

(*i*) *Rubrophen* : This is a dye which has been claimed to be of some value in the treatment of leprosy but we were unable to confirm this. None of the cases chosen showed any improvement after the administration of the drug. The experiment was discontinued as no further patients were willing to volunteer.

(*ii*) *Fouadin* : During the year a small study of lepra reaction was undertaken with a view to determining the reason why various workers reported failure to control this condition with the antimony products. It was concluded that the confusion in the literature was due to the inadequate appreciation of what lepra reaction really meant, and that frequently what we, for want of a better name, describe as the reactive state (an allergic condition seen in tuberculoid leprosy) was confused with true lepra reaction, and further lepra reaction could itself be subdivided into acute, sub-acute and chronic stages. The antimony products were only of definite value in acute lepra reaction bringing the temperature down in all cases after 4-6 injections. In the course of this experiment it was found that the pentavalent antimony product fouadin was as effective as pot. antimony tartrate and easier of administration.

Although the modern advances in the therapeutics of leprosy commenced more than 15 years ago the optimum dose of the remedy has to our knowledge, never been adequately investigated. We are of opinion that large doses above 10 c.cs. are of more value than small doses. An investigation has therefore been undertaken with a view to determining the optimum dose of the hydnocarpus remedies. All the adult males in the Lady Willingdon Leper Settlement (approximately 400) have been divided into three groups. Group A : receive 1-10 c.cs. of the hydnocarpus preparation a week, Group B : 10-15 c.cs. and Group C : 15-25 c.cs. The selection is a random selection. Patients when they are admitted are placed in the low, medium and high groups respectively, in order of admission irrespective of their type of leprosy. Such an experiment must be continued for 18 months or 2 years before results can be analysed. This investigation is proceeding.

Clinical investigation.

An intensive study of the various types of cases seen in South India is being undertaken. A study of tuberculoid leprosy has been taken up and an answer to whether tuberculoid leprosy tends to pass into the more serious lepromatous variety sought. Some authorities believe that this occurs, but evidence accumulating up to now tends to show that in South India at least, frank tuberculoid leprosy remains true to type and does not become lepromatous. All cases which have appeared to have become lepromatous seem to fall into an intermediate category, which have been termed intermediate or border line cases and are not true tuberculoid. These cases can usually be recognized clinically and have up to now all been leprolin negative, whereas a typical tuberculoid leprosy is always strongly positive. In addition these cases present a fairly consistent histological picture the main features of which are as follows :

(1) Uniform and massive infiltration beneath epidermis respecting the sub-epidermal zone as in leproma.

(2) Epithelioid cells are present but not in the characteristic formation seen in frank tuberculoid leprosy, occasionally small giant cells are present.

(3) Foamy cells may be seen but these are not numerous and usually only in certain parts of the section.

We believe it is the occurrence of this group of cases which has encouraged the view that tuberculoid leprosy occasionally becomes lepromatous.

Field investigation.

This has followed two definite lines :—

(1) An epidemiological study of lesions in children.

(2) A detailed study of leprosy in a limited rural area with a view to devising a scheme of village control.

(1) An intensive study of child leprosy has been continued in Saidapet and we believe that there is considerable evidence indicating that the more serious type of leprosy develops where contact is maximal and that the most important form of contact is intrafamilial. The following tables demonstrate this :

Table V.

Cases in which the infection was traced to an open case.

	Intrafamilial				Extrafamilial	
	Room contact		House contact		Street contact	
	No.	%	No.	%	No.	%
Total neural cases	51	44.0%	22	19.0%	42	36.1%
Incipient lesions of leprosy in children	14	66.7%	2	9.5%	5	23.8%
Lepromatous	27	61.4%	1	2.3%	16	36.3%

It is further interesting to note that the percentage of traceable open contacts has a direct relationship to the severity of the disease as shown by the following table :

Types	No. of cases showing *untraced open* contacts. No.	%
N1—Simple neural	51	43.6%
N1— Minor tuberculoid ..	58	45.3%
N1—Major tuberculoid ..	20	51.3%
Incipient lesions of leprosy in children	2	8.0%
Lepromatous	21	30.9%

In order further to demonstrate this, all neural type lesions have been divided into those with single lesions and those showing multiple lesions,

A single lesion means not more than two lesions and the difference between intrafamilial and extrafamilial contact is striking.

Table VII.

Percentage of Intrafamilial Contacts.

Type	Total No.	Intrafamilial contact	Percentage
Neural :			
Single lesions ..	41	23	19.8%
Multiple lesions ..	75	50	43.1%

There also appears to be a definite tendency for the increase of the *sex-rate** etc. in leprosy in the older age groups and confirms the observation that leprosy tends to develop most often before puberty.

Table VIII.

Age Group of Children Under Observation.

Total	0—4	5—9	10—14
304	14.1%	51.69%	33.2%

Age Group of Children Under Treatment.

Total	0—4	5—9	10—14
137	2.1%	27%	71.5%

The following tables indicated the sex-rate in varying age groups at the Silver Jubilee Children's Clinic, Saidapet.

Table IX.

Children under Observation.

	0—4 Yrs.	5—9 Yrs.	10—14 Yrs.
Males ..	55% (approx.)	52%	74%

Children under Treatment.

Males ..	group too small to be significant.	65%	72%

Table X.

Sex-Rate according to severity of disease in all age groups.
(0—14)

	Males
Doubtful lesions	48%
Definite but slight lesions (three monthly observation) ..	61%
Definite but more pronounced lesions (monthly observation) ..	62%
Closed cases (Under treatment)	69%
Open cases (Under treatment)	73%

* *Sex-Rate :* As defind by the Cairo Conference (1938) is the number of male cases per 100 cases of leprosy.

These figures give support to the contention that the sex-rate is more nearly equal in the younger age groups, and that there is also evidence that males suffer more frequently from serious leprosy than females, as witnessed by the difference between 61% in males in the group with mild lesions and 73% in the group with severe lesions.

Our investigation of a type of lesion termed the 'incipient lesion' has been undertaken. It is felt that evidence is accumulating to show that these lesions are the precursors of lepromatous leprosy, and further that in addition to this type of case the simple neural lesions in childhood are very liable to become lepromatous in adult life. It is impossible to summarise these investigations but a communication will be submitted for publication in due course.

(2) An area some 23 miles from Chingleput has been chosen for an intensive study of leprosy in a group of villages and an investigation centre and a control unit have been established. This investigation has been in full working order only for six months. The following table gives a summary of the survey in the largest village of the area and its cheri.

Table XI.

Polambakkam Cheri : Adults and Children.

		Males	Females	Total
1.	Total population	239	245	484
2.	No. examined	210	226	436
3.	No. suffering from leprosy ..	18	10	28
4.	Gross incidence	64.2 per 1000		
5.	Sex incidence	85.7 per 1000	44.2 per 1000	
6.	Sex-rate	64.3%		
7.	Open case rate	17.85%		
8.	Child rate	28.6%		

20 persons left temporarily for other villages
22 persons left temporarily for Madras for work.
3 persons left permanently.
3 unaccounted for.
48 Total.

Table XII.

Polambakkam Village : Adults & Children.

		Males	% examined	Females	% examined	Total
1.	Total population	362	98.6%	372	97.4%	734
2.	No. examined	357		362		719
3.	No. suffering from leprosy	22		17		39
4.	Gross incidence			54.24 per 1000		
5.	Sex incidence	61.62 per 1000		49.72 per 1000		
6.	Sex-rate			56.4%		
7.	Case type rate			25.6%		
8.	Childhood rate			25.6%		

Reasons for discrepancy in the figures of the total population and the number examined,:

5 males remain to be examined who have temporarily left the village.

10 females remain to be examined who have temporarily left the village.

A number of questions arise from this work, and the answer to the following problems may be forthcoming when all the villages in the group have been studied and observed for a period of years :

(1) How heavy must the incidence of leprosy be in a village for the disease to spread and become a serious problem ?

(2) Is there any relationship between the open case rate and child rate and the incidence of the disease ?

(3) How far do intrafamilial and extrafamilial contact play in the spread of the disease ?

Animal experiments.

Recent reports have indicated that it is possible to infect Syrian hamsters with human leprosy after the removal of the spleen. Since the main defence in human leprosy appears to be the cells of the reticulo-endothelial system, it was thought that splenectomy might be a vital factor in producing the infection in animals. Further the Syrian hamster is a small animal and it would be advantagous to find a larger animal capable of being infected with leprosy. It was therefore decided to work with monkeys (*M. sinicus*). This investigation has been proceeding for more than 18 months. The technique adopted is to splenectomise the monkey and insert a nodule, taken recently from a patient, into the peritoneum anchoring it to the splenic stump. The nodule is then fixed to the peritoneum so as to bring the infected material in direct contact with the abdominal wall and thus encourage lymphatic spread.

The first series of experiments consisted of seven animals and the following were the preliminary conclusions :—

(1) There appears to be a possibility of infecting a monkey by performing preliminary splenectomy and embedding a nodule in the peritoneum, preferably fixing it to the splenic stump.

(2) There may be a greater chance of success if the animal is splenectomised two months after insertion of the nodule intra-abdominally, with reinoculation at the time of the second operation.

(3) The experiments have revealed the possibility of individual variability of the factor of resistance, and many monkeys may have to be used before one is found that is actually susceptible to the infection. In one monkey of the series there was evidence of dissemination of the disease, as bacilli were found in the liver, omentum and in ulcers of the abdominal wall on post-mortem.

These experiments are being continued and investigations into the question of individual variability of the factor of resistance is being further studied. Attempts are being made to block the reticulo-endothelial system after splenectomy in the hope that this will result in an animal becoming infected. This work is undertaken in co-operation with Drs. C. G. Pandit and K. P. Menon at the King Institute of Preventive Medicine, Guindy, with the kind support and encouragement of Lt.-Col. Shortt, Director of the Institute.

PLAGUE.

29. Plague researches under Lt.-Col. S. S. Sokhey, I.M.S., at the Haffkine Institute, Bombay.

I. Serology of Plague.

Serological reactions—agglutination and precipitation—have long been used for diagnostic purposes and for measuring immunity against any particular infection. But a glance at the literature on the subject will show that the relationship of agglutination and precipitation with immunity is not yet clear. Agglutination titres give more an indication of infection than a quantitative measure of the immunity developed in the host due to infection or inoculation. With precipitins the case is however, somewhat different. Wherever it has been possible to get a definite and well defined antigen as in the cases of pneumococcus or meningococcus, the relationship between precipitins and protective potency of antisera has been shown to be a quantitative one. This has been mainly due to the use of an exact quantitative method of measuring the amount of precipitins present in a serum in terms of the nitrogen in the specific precipitate.

For a good comparison of serological reactions with protective power of an anti-serum, it is necessary that besides having good and reliable techniques for serological tests, one should have an experimental animal susceptible to the organism under study and a quantitative biological method for determining the protective potency. The availability of an animal of choice for *P. pestis* work and also a very precise biological method for measuring the protective power of an anti-serum provided an excellent opportunity of examining the value of these serological reactions as a measure of immunity. Moreover, the establishment of an *in vitro* serological method having a quantitative relationship with the protective potency would be of the highest value in the production of serum as it would enable the progressive increase of immunity to be measured easily from week to week and permit of the horses being bled at the right time. Therefore, this year the work was carried out along the following lines.

(1) agglutination reaction in *P. pestis*.

(2) fractionation of the immunising antigen or antigens present in the Haffkine vaccine.

(3) quantitative estimation of the precipitable antibodies present in *P. pestis* antisera.

(1) *Agglutination.* After a prolonged investigation it was found that an antigen prepared by washing 72 hours' growth (at 32° C or 37° C) on agar (pH 6.8) with half normal saline made alkaline (pH about 8.0) with NaOH gave the best results. About 20 c.c. of alkaline half normal saline was used for each Roux bottle and the washing was transferred to a sterile conical flask containing glass beads. It was shaken for two or three minutes, filtered through sterile cotton wool and the filtrate diluted with half normal neutral saline to an opacity equivalent to that of Brown's opacity tubes 2 or 3. The final pH of the emulsion was about 7.6. It is expected that the pH used here will not appreciably deteriorate the antigen or antigens responsible for protection in *P. pestis* in view of the fact that although in the case of Haffkine vaccine the *P. pestis* growth

remains in contact with a pH of about 8.3 for over a week, the antigenic property as far as protection against plague is concerned remains intact.

Results obtained with these antigens have been found to be repeatable, but the titres obtained did neither show any relationship with the progress in immunisation of the animal nor did they show any relationship with the protective power of the plague antisera in mice. This will be clear from the fact that antisera against avirulent strains which showed little or no protection gave high agglutination titres whereas, those raised against virulent strains and showed definite protective power, gave low agglutination titres. Two sera of about the same mouse protective dose did not show the same agglutination titres. Thus it can be said that the results obtained so far show that agglutination titres measured according to the method used here cannot be taken to be a measure of the immunity developed in an animal against *P. pestis*.

2. *Immunising antigen in Haffkine vaccine.* Haffkine vaccine filtrate which contains almost the whole of the protective substance of the vaccine, was used for these experiments. The whole of the protective substance could be precipitated out with dilute acetic acid, because the supernatant fluid did not show any protective power. This precipitate could be fractionated with sodium sulphate into different fractions with varying protective values. The work is proceeding but one thing is clear that it is the protein alone that is responsible for the immunising value of the Haffkine vaccine. Polysaccharides do not seem to play any significant role.

3. *Quantitative estimation of precipitin.* Haffkine vaccine filtrate and solutions of the protein fractions in (2) above were used as antigens, to measure the amount of precipitable antibodies present in the antiplague sera of horses produced in the Institute for therapeutic purposes. Some of the rabbit sera produced for our work were also used. The amount of N_2 present in the precipitate when the supernatant showed a slight excess of antigen, was taken as a measure of the precipitable antibodies present in the sera.

The amount of precipitin nitrogen increased in the serum of the animal with the progress of immunisation. It was very easy to distinguish a bad serum from a good one because the former invariably contained a very low amount of precipitin nitrogen in it. But the real trouble came in when two good sera had to be compared. The estimations of precipitin nitrogen could not be of any use in such cases. Thus so far it has not been possible to establish any quantitative relationship between precipitins and protection.

From the above it may be inferred that in these reactions one may be dealing with other antigens or antibodies besides those responsbile for protection, and the results obtained may be due to the interaction of all these. In order to get a quantitative relationship we should be able to distinguish the protecting antigen from the rest, and that may be possible to do by fractionating the Haffkine vaccine filtrate. This work is now in progress.

II. Chemo-therapy.

Using Haffkine-Institute-inbred-mice and the standardised infective dose three preparations—prontosil rubrum, prontosil soluble and M & B 693

were tried. The prontosil preparations were found to have no curative action whatsoever even when the administration of the drug was commenced at the same time as the induction of infection.

M & B 693 also had no curative action when given in ten times the therapeutic dosage, *i.e.* 50 mg. per kilo weight. But when 50 times the therapeutic dose was given *i.e.* 1 mg. per gram of body weight, a certain amount of curative action was noticed up to 48 hours after infection. Thereafter even this large dose did not prove effective. Further work is proceeding especially on the combined action of serum and M & B 693.

III. Serum field trials.

The Madras Government has built a plague hospital at Cumbum Valley and it is proposed to send a unit down to the place as soon as plague cases start coming in. Twelve horses are being immunized for the purpose.

IV. Plague vaccine.

The study of the factors which influence the preparation of an effective plague vaccine was continued.

1. *Optimal temperature for the growth of the plague bacillus.* Literature has nothing to say as to the exact optimal temperature for the growth of this organism. Using the method of colony counts described elsewhere, studies were therefore made to determine the optimal temperature of growth. It was found that maximal growth in broth took place on a broad zone of 23° C to 27° C. Growth at 37° C was found to be less than one-tenth of the growth at 23°-27° C and at 41° C the organisms entirely died out in four days. On the lower side of the scale perceptible amount of growth appeared at as low a temperature as 4° C.

2. *The effect of temperature of incubation on the resultant vaccine.* Broth vaccines were incubated for four weeks at 27° C, 32° C and 37° C. It was found that the protective power of the vaccine varied in the order mentioned, 27° C vaccine being the most potent. Further experiments with vaccines incubated at 23° and 25° are being carried out.

3. *Optimal pH for the growth of the plague bacillus.* This question has also been studied and it is found that maximal growth in broth takes place at 6.4 pH, but the zone of maximal growth is fairly wide, 6.2 pH to 6.8 pH. While growth can still take place on the acid side at as low a pH as 5.0, the growth comes to an end at 9.0 pH.

4. *The effect of buffering the medium on the resultant vaccine.* When the organism is incubated in ordinary broth with a pH of 6.8 for four weeks, the pH of the medium rises to 8.4 and the growth comes to an end. It is conceivable that buffering of the medium may permit of better growth. Vaccines so made are under test.

5. *Agar and broth vaccines compared.* It was found and reported before that broth vaccines incubated at 27° C had higher protective power than broth vaccines incubated at 32° C, while in the case of agar growth vaccine those incubated at 37° C were more potent than those grown at 27° C. A study was undertaken to compare broth vaccines incubated at 27° C with agar vaccines incubated at 37° C. The work is still unfinished but the results of 11 determinations show that the portective power of the two vaccines is about equal, with slight advantage in favour of broth vaccine

from the point of view of preparation on a large scale. Further studies on this subject have been suspended until all the factors which influence the growth of the organism have been determined.

30. Enquiry into the recrudescence of plague in the Cumbum Valley under the Director of Public Health, Madras.

Plague in Cumbum Valley.

Plague infection was unusually mild during the year, only 86 deaths having been reported till the end of September as against 368 deaths during the corresponding period of the previous year. Since the middle of March, only a few sporadic cases were reported. There is thus every indication that if the experimental measures could possibly be continued unabated, the endemic plague of the Cumbum Valley is bound to clear up in the near future. During March there was a limited outbreak of pneumonic plague with 12 attacks and 12 deaths in one of the villages, all among the immediate contacts of an imported case from a distant village.

Cyanogas fumigation.

Thirteen villages and hamlets were fumigated during the period. A total of 131,785 rat holes and 546 cracks in walls in 14,642 houses, in addition to the complete fumigation of many of the infected houses and most of the petty grocery shops, godowns, etc., were fumigated. Two thousand six hundred and thirty-three *R. rattus*, 115 babdicoots, 8,922 house mice, 38 musk rats and 3 snakes were picked up during these operations. Nine thousand four hundred and ninety-nine of these rodents were examined in the laboratory and five proved positive. One hundred and two carcasses reckoned as rat falls in various villages were examined and 54 were found positive. From Periyakulam Municipal town adjoining Cumbum Valley, 13 dead squirrels were also examined, of which 8 proved positive to plague. The fleas recovered from these squirrels were found to be Ceratophyllus arcotus.

The results of fumigation were uniformly encouraging in all the villages, specially where there was imminent threat of spread of infection by contiguity. A few selected villages are being periodically fumigated irrespective of plague, to see if the rat population so depleted will serve to keep off plague.

In about 400 houses both cyanogas fumigation of rat burrows and rat trapping were done, one following the other, to note the extent of rat infestation in houses apart from rat burrows. One elongated improved wonder trap was set for each room of every house. It was found that for every rat which was trapped after fumigation about 6 were killed by fumigation. The actual infestation of rats in roofs is being further studied.

Effect of fumigation on rat and flea densities.

These observations were continued during the year. It has already been reported that while rat density is reduced by 40-80% by fumigation, flea index continues much the same, as judged by the prevalence of fleas on the surviving rats. Every rat nest is a prolific nursery of fleas even after their fumigation inside rat holes. Unless, alongside fumigation, attempt is also made to search out rat nests and destroy them, preferably by burning them off, it is not possible to produce any obvious reduction in the flea index of rats.

Study of the prevalence of fleas apart from rats.

Studies of rat nests were continued and have now provided sufficient data to estimate the relative bearing of various environmental and meteorological factors on the biology of X. astia and X. cheopis fleas. One hundred and twenty-seven rat nests as recovered from all their usual sites and during different seasons of the year have so far been studied. Two thousand eight hundred and twenty-two parental fleas and 14,587 of their first filial generation have thus been observed. Considering all seasons of Cumbum Valley, X. astia comprises of 52 to 74% of the total fleas (the rest being all cheopis) in nests from underground rat holes. Similarly in nests from rat holes in mud walls, X. cheopis forms from 62 to 86% of all fleas. In nests from cracks in mud walls, X. cheopis forms from 60 to 95% of all fleas. In nests from various harbourages on the surface of mud floors, X. cheopis forms from 55 to 82% of all fleas. In nests from thatch roofs, X. cheopis forms from 66 to 91% of all fleas. Thus it is obvious from these biological observations that under natural conditions, X. astia prevails best during all seasons of Cumbum Valley in the environment of underground rat holes, while cheopis outnumbers astia in the same premises under the more variable meteorological conditions of floor surfaces, walls, roofs, etc. These observations may thus form yet another approach to the study of the biology of fleas in the field, where elaborate laboratory facilities for controlled observations outlined by Prof. Buxton (1938) are not available.

Investigation of Sylvatic plague.

Attempt has been made in previous years to see if sylvatic plague prevails in the Cumbum Valley, but no definite evidence pointing to its existence has so far been obtained. Parallel studies were made during the year (May-July) in the Nilgiris district, working in the Pasteur Institute, Coonoor. Over 1,000 rodents and about 2,000 fleas were examined for the purpose. The chief prevailing field rodent here is *Gunomys kok* and the fleas which largely prevail on them are *Ceratophyllus nilgiriensis* around Ootacamund (7,300 ft.) and *stivalius* (3 or more species—*ahalae, sporous, ferinus*) around Coonoor (5,900 ft.). No definite evidence of plague infection was obtained from the rodents or fleas collected. The susceptibility of these rodents to plague and the vector efficiency of those fleas were also studied. Gunomys rats of Nilgiris are highly susceptible to plague and these abound in the potato farms and elsewhere. Employing white mice as test animals, it was found that stivalius fleas can transmit plague quite well. Fleas fed three days earlier on septicaemic mice were employed and these successfully transmitted the infection. Longer periods were not tried for want of time. Similarly with *Ceratophyllus* fleas, though

the test animals died prematurely, the fleas themselves showed live and virulent plague bacilli in their stomachs after a week of feeding on plague infected mice.

A FEW OBSERVATIONS ON THE HABITS OF RATS.

The following observations were made employing the rats which had been continuously left at large for long periods in the model house described below.

1. The maximum vertical height which house rats (*R. rattus*) could clear was found to be not more than 2 feet.

2. Rats could sustain themselves on dry paddy alone without any water up to 24 days.

3. Rats could sustain on water alone for 10 days.

4. Rats could live up to 6 days without any food or even water.

5. An average rat during the course of an year could eat as much as 3,908 grammes of paddy and drink about 143½ ounces of water (6 rats during 53 days ate up 3,405 grammes of paddy and drank 125 ounces of water, when ample quantities of both were supplied. Their air dried excreta weighed 217 grammes.)

MODEL RAT-PROOF MUD HOUSE FOR CUMBUM VALLEY.

The construction of a model hut has already been reported on last year, but the observations in this hut may now be said to be complete for one cycle of seasons in Cumbum Valley.

The design of a cheap house which will eliminate rat harbourages has been a long felt need for Cumbum Valley. The familiar type here is the thatch roofed mud house. Unsplit bamboos are largely used for the wood work and a thick bed of straw (cholam or paddy) over a single layer of woven cocoanut palm leaves form the roof cover. Walls and floor are of beaten earth. Thus it needs no verification to believe that in every aspect, this type of construction is defective and has to be rectified without materially increasing the cost. Sand, lime stone, straw and bamboos are locally available. In brief the special features of a rat-proof house, as based on the experience of the model house referred to above, may be stated as follows :—

(1) *Floor.* A plinth height of 1½ ft. should be provided. Three inches of rammed lime stone mortar, over 1¼ ft. of sand filling, will form a strong enough basement and the floor may be plastered over with cement mortar or mud mixed with sand.

(2) *Walls.* The lower ½-1 ft. and the top ½-1 ft. of the walls should be built of brick in mortar and the rest with a mud and sand mixture in equal proportion. One layer of bricks should be inlaid on the floor all along the outer and inner walls, so as to avoid burrowing of rats at the joints. The corners of walls should be rounded inside and outside. The surface of the walls, inside and outside, should be smoothly plastered, preferably with a mud mortar with antheap earth, and white washed.

(3) Above the door height, a continuous 9"-12" projecting ledge should be introduced on the inner walls. This ledge should ordinarily ensure the thatch roof free of rats and also serve as a shelf for sundry household articles. On the outer wall also a similar ledge should be provided, at least over the top of all windows and doors and projecting a foot from either ends of each.

(4) Doors and windows should be guarded against gnawing of rats by nailing on metallic strips about 3" to 4" in width, along the lower edge.

(5) *Roof.* Only split bamboos should be used for rafters and beams and these should be so fixed so as to have the open hollow exposed to view and in a way that dead spaces, where rats can shelter, will be avoided.

The model house constructed in Cumbum has now stood the test of several months with both rooms artificially infested each with a dozen rats throughout. The floor as originally constructed was burrowed through and had to be replaced as advised above. The walls have not cracked so far. No rats have so far skipped the ledges in the room provided with continuous ledge, but in the other room where only corner plates and ledges over windows and doors were provided, a few rats actually skipped the ledges and escaped through the roof. The approximate cost of constructing such a house according to local rates will be about Rs. 75/-.

RABIES.

31. Antirabic vaccine enquiry under the Director, Pasteur Institute of India, Kasauli.

I. HUMAN EXPERIMENTS.

Experiments to test the value of immune serum in antirabic treatment.

The investigation into the value of antirabic serum as an adjunct to treatment with a carbolized dead vaccine was continued. The results from the commencement of the investigation up to 31st December 1938 are given below :—

(1) Treated at the Pasteur Institute, Kasauli.
Total treated with serum plus vaccine 1,433.
Deaths 23. Death rate 1.61.
Total controls treated with vaccine alone 743.
Deaths 18. Death rate 2.42.

(2) Treated at selected Centres in the Punjab and the United Provinces.

Total treated with serum plus vaccine 2,360.
Deaths 42. Death rate 1.78.
Total controls treated with vaccine alone 5,421.
Deaths 68. Death rate 1.25.

Like the figures reported last year the results indicate an advantage in the use of serum in the case of institute-treated cases but not in the cases treated at centres.

The experimental use of serum was stopped in the Punjab on May 26th, 1938, and in the United Provinces on August 31st, 1939.

The figures for the United Provinces for 1939 remain to be collected.

It has not yet been proved satisfactorily that the use of serum in addition to vaccine is an advantage in antirabic treatment.

II. ANIMAL EXPERIMENTS.

Experiments in connection with the therapeutic value of animal vaccine.

Experiments were undertaken to compare the pre-infectional immunizing value of carbolised 6% Paris sheep vaccine manufactured by this Institute for the treatment of animals against that of a proprietary vaccine which is on the Indian market.

Dogs and monkeys were used. Fifteen dogs and 25 monkeys were treated with the Institute vaccine. The dose administered was 5 c.c. for 7 days. On the last day of the above treatment, a second group of 15 dogs and 25 monkeys were inoculated with one dose of the proprietary vaccine. The dosage administered was that recommended by the manufacturers for prophylactic immunization, *i.e.*, 5 c.c.

Eighty-two days after completion of treatment, the surviving animals in both groups, together with a third group of controls, *i.e.*, 15 dogs and 23 monkeys, were infected in the muscles of the neck with street virus.

The infecting dose was 2 c.c. of 2% emulsion. The results are tabulated below :—

Dogs

	Total infected	Died of other causes	Died of rabies	Escaped infection	Percentage deaths from rabies excluding deaths from other causes
Institute vaccine	14	1	2	11	15.4
Proprietary ,,	14	4	6	4	60.3
Controls	15	0	2	13	13.4

The experiment was not satisfactory as the infecting street virus used was of low virulence.

Monkeys

	Total infected	Died of other causes	Died of rabies	Escaped infection	Percentage deaths from rabies excluding deaths from other causes
Institute vaccine	23	0	1	22	4.3
Proprietary ,,	23	0	15	8	65.2
Controls	23	1	13	9	59.1

The experiment was not very satisfactory as the infecting street virus used was of low virulence. For what they are worth the figures suggest that the proprietary vaccine had little protective value while the Institute vaccine was of considerable protective value.

Experiment to ascertain the antigenic value, if any, of antirabic vaccine that has become "time-expired".

Three groups of 18 guinea-pigs each were treated as under :—
Group I. with 6 weeks old carbolised 5% vaccine.
,, II. with 8 months old carbolised 5% vaccine.
,, III. with one year old carbolised 5% vaccine.

The daily dose of vaccine was 1 c.c. for 14 days.

Eighty-two days after completion of treatment, the surviving animals together with a fourth group of 10 controls were infected in the muscles of the neck with street virus. The infecting dose was 1 c.c. of a 2% emulsion.

	Total infected	Died of other causes	Died of rabies	Escaped infection	Percentage deaths from rabies excluding deaths from other causes
Six weeks	8	2	4	2	66.6
Eight months	10	2	4	4	50.0
One year	9	1	5	3	62.5
Controls	10	0	10	—	100.0

The experiment suggests that the vaccine does not lose any of its antigenic value after storage for one year. The numbers are of course rather small, partly on account of the high mortality from other causes prior to infection.

TUBERCULOSIS.

32. Tuberculosis enquiry under Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta.

TUBERCULOSIS IN RELATION TO INDUSTRY.

The procedures of investigation and the methods of approach, by investigating a "Sample" first and then a general "Serial" survey of the workers, were already mentioned in the previous year's report. A summary of the findings of the "Sample survey" was also noted therein. The data are being further analysed for final publication. The following is a review of the findings on the "Serial examination and tuberculin testing of all workers".

SERIAL EXAMINATION OF ALL WORKERS.

To date, 3008 workers have been examined, and during the year under review, 1858 workers have been examined. The data obtained in 3008 workers are outlined below :—

Sex :—Males : 2589, Females : 419.

Age groups :—20=15, —25=216, —30=697, —35=820, —40=665, —45=343, —50=158, —60=86, 60+=9.

It will be seen that quite a large number consists of old workers. The majority belonged to the age group 26-40 years.

Religion : Hindus—1994, Mahomedans—1013, Indian Christian—1.

Original domicile according to Province : Bihar—1559, C. P.—491, U. P.—332, Orissa—240, Bengal—207, Madras—179. It will be seen that the vast majority of workers are drawn from distant places. So it is likely that the effects of change of environment and climate have been considerable among these workers.

According to departments of the industry : batching—303, preparing—500, spinning—570, winding—271, beaming—141, weaving—1170, finishing—53.

GENERAL INCIDENCE OF TUBERCULOUS INFECTION.

All the 3008 workers were Mantoux tested. 89.3% gave a positive reaction and 10.7% a negative reaction. The population is thus seen to be highly tuberculised—a rate of infection much higher than what was found by us some years ago in urban areas (55.1%).

The intensity of reaction varied as follows : 23.8% reacted "one plus", 28.3% "two plus", 28.9% "three plus" and 8.3% "four plus". Counting "three and four plus" as "high reactors", the percentage of such workers comes up to 37.2—a very high rate. Such intense reactors are liable to develop acute exudative type of lesions if and when they fall a prey to this disease. These cases in particular should be followed up through several years.

General incidence of 'active' and 'arrested' tuberculosis.

For such findings, radiological examinations are essential. It was hardly possible, for financial reasons, to include the total population of the

mill for radiological examination. The past history, history of industrial life, housing, financial condition, medical history etc. were ascertained in respect of every worker and they were submitted to an uniform and careful physical examination and tuberculin testing. Sputa were examined wherever available. On the findings of these examinations, only those workers were selected for X-ray examination who presented suspicious physical findings, symptoms of disease or both and/or who exhibited high tuberculin reaction. Ninety-five workers were found to belong to the former and 1119 workers to the latter group, making a total of 1214 workers earmarked for X-ray examination. Consecutive and regular radiological examination was prevented by long and serious labour disturbances and, to-date, only 358 workers of this group could be X-rayed. Among these, 8.4% showed 'definite' radiological evidence of active pulmonary tuberculosis and in 6.1% such lesions were considered to be highly 'probable', *i.e.* in 14.5% evidence of active disease could be elicited. This is an exceptionally high rate, although in a highly selected group. If the rate of disease incidence in the selected group of these 1214 workers be distributed over the total workers hitherto examined (3008) the rate of definite and probable cases comes to 5.8%. This is definitely a high incidence of disease among the population.

Evidence of arrested lesions was detected in 72.9% of the workers.

Incidence of tuberculous infection and disease in relation to certain general factors.

Influence of age.

Infection—It has been found that the frequency of infection is almost equally distributed over all the age-groups, except in the very old. The intensity of reaction, on the other hand, predominated in the age period 35-50 years (*e.g.*—40=41%, —45=46.9% and —50=40.6%).

Disease—The highest percentage of "definite" (13.6) and 'probable' (9) disease was detected in the age group —40-45. It is quite possible that younger workers who get a more acute type of disease, are seeded out from the industry and return home. These are the people who may be responsible for carrying and disseminating disease around their village homes. Those who have acquired some degree of tolerance to the disease have continued to work and have been detected during our investigation. The percentage of arrested disease, however, is almost equally distributed over all the age periods.

According to province.

Infection—The rate of infection has not been found to vary appreciably among workers of different provinces, indicating that probably their long stay in the industrial area has made them homogeneous in this respect. The strong reactors were, however, found to be largest among the Biharis (*i.e.* 43.2%).

Disease—The highest percentage of definitely tuberculous cases (16.6%) and that of 'definite' and 'probable' cases (33.2%) was found among C. P. workers. Bengal ranks second with (26.5%) 26.5%. Madras produced the lowest figure (9.1%). The highest percentage of 'arrested' lesions was detected among the workers from Madras (90.9%) and the lowest among C. P. workers (54.2%), pointing out that the workers of the province who

show the lowest ratio of 'arrested' lesions show the highest incidence of active disease and vice versa.

According to sex.

Infection—The rate of infection in both sexes is almost the same. Females showed a higher percentage of mild reactors (68.9%) and males of strong reactors (39.3%).

Disease :—Due to labour and other disturbances, the number of females X-rayed is very much less than males ; so a reasonably comparative study is not possible. In the males, 8.5% were detected to be 'definite', 5.6% 'probable' and 73.4% 'arrested' pulmonary tuberculosis.

According to Religion.

Infection—The percentage of reactors and the intensity of reaction were found to be almost identical among Hindus and Mahomedans.

Disease—Definitely 'active' tuberculosis was detected in greater percentage among the Mahomedans (12.5%) than in Hindus (4.9%) whereas the percentage of 'probable' disease in Mahomedans was 4.4% and in Hindus 7.4%. Arrested disease is almost equally distributed in both.

According to sections of the industry.

Infection—Not much difference in rate of infection could be detected among the workers of the different departments. The "winding" department produced the highest percentage of strong reactors (51.7%) and the "preparing" the lowest (16.1%).

Disease—The highest percentage of 'definitely' active cases was detected in the 'winding' department (12.1%) but the total percentage of 'definite' and 'probable' cases was highest in the batching department (27.2%). The beaming department showed the highest number of 'arrested' cases (86.2%).

OTHER FINDINGS.

Evidence of bronchitis of a mild degree, was detected in 15.5%. Probably the high concentration of dust in the working atmosphere is responsible for this condition, but it is striking that hardly any disability has been produced thereby. "Jute dermatitis" was detected in 8.4%. Such high figures in the incidence of bronchitis and dermatitis, coupled with an average high eosinophilic count of blood in the sample survey, might indicate that jute dust probably produces some kind of 'allergy' causing these manifestations. Naso-pharyngeal catarrh was detected in 2.4% only. To ascertain the incidence of non-tuberculous lung diseases (esp. pneumonia) prior to and after the entry of workers into industrial life, the history of past illnesses was carefully elicited. Such conditions were detected in 20 workers prior to their entry into industrial life and in 68 after it, which might indicate that industrial life probably makes this hazard more frequent among the workers in this industry.

HOME SURVEY.

One thousand two hundred and seventy-two dwelling houses, both in the barracks (1022) built by the mill authorities and in private bustees (220) and villages (30) in the neighbourhood, were surveyed. This included a survey of the house and its surroundings, the social customs

and habits, finance, nutrition, general mortality and tuberculosis morbidity and mortality. The findings were recorded in specially prepared schedules.

It was generally noticed that sanitation was much better in the barracks built by the mill authorities than in the private bustees. The mill authorities take special care on almost all aspects of public health, yet the ignorance and unhygienic habits of the workers make their homes unhealthy in such a way that, in spite of the care of the authorities, the general sanitation remains bad. The rooms rarely have cross ventilation. There are many customs which seem to definitely help in the spread of infection. As was noted before, a considerable number of workers are addicted to alcohol and other narcotics. This and other causes make them bear a burden of heavy debt and interest. A survey of nutrition revealed that the diet of the workers, in the majority of cases, although almost adequate in calorie value, was grossly unbalanced.

Contact Survey—Fifteen houses, belonging to 6 'definite' and 9 'probable' cases, have hitherto been surveyed. Among the contacts, 18 were children. All of them reacted positively to tuberculin, 5 giving a mild and 13 strong reaction. Of 38 adult contacts, all reacted positively to tuberculin, 31 mildly and 7 strongly.

Tuberculosis in "home contacts" (children).

To date, 917 children of 15 years and under have been examined. An analysis of these children is given below :—

age : 0-2 yrs =129 ; 2-5 yrs =242 ; 5-10 yrs =365 ; 10-15 yrs =181.
sex : males—520 ; females—397.

religion : Hindus—596 ; Mahomedans—160 ; Christians—127 and others—34.

Nature of contact.

(*a*) source :—'open'—695, 'closed'—103 and 'unknown sputum'—119.

(*b*) closeness of contact : intimate—824, distant—93.

(*c*) continuity of contact : immediate—630, recent—237, remote—50.

(*d*) duration of contact : for 1 yr—255, 2 yrs—215, 3 yrs—80, more than 3 yrs—24.

Nature of examinations done.

Both Mantoux test and radiological examination done—605; Mantoux test alone—824 ; radiological examination alone—698.

General incidence of tuberculous infection and disease (active and arrested).

The total incidence of positive reactors—67.9%.

The total incidence of demonstrable X-ray lesions was 46.7% (11.6% 'active lesion', 6% 'arrested lesion' and 29% tracheo-bronchial adenopathy).

It appears that the rates of disease and infection differ very little between the different sexes and communities. It further appears that, in general, the infection rate is 21% higher than the rate of demonstrable evidence of disease.

Findings on tuberculous infection and disease (active and arrested) in relation to some important factors.

1. According to age.

Infection—The percentage of positive was found to vary directly with age.

Disease—It is seen that the incidence of 'active' parenchymal lesions increases with age up to 10 years and then decreases rapidly. The incidence of 'arrested' lesions increases, while that of tracheo-bronchial adenopathy decreases with age.

The average incidence of demonstrable X-ray evidence of disease varies inversely with age. In each age group upto 10 years the incidence of 'active' lesions is higher than that of 'arrested' lesions.

2. According to nature of contact.

(a) Source.

Infection—The percentage of positive reactors in the 'unknown sputum' group was found to be highest (71%). The incidence of strong reactions (3+ and above) was highest in the 'open' source series, while that of mild reactions (1+) was highest in the 'closed' group. In the 'open' source group, the incidence of infection has been found to increase with age and is highest among Mahomedans.

Disease—The incidence was highest in the 'unknown sputum' group.

(b) Closeness of contact.

Infection—Intimate contacts showed a much higher percentage of reactors (70.3%) than the distant contacts (47%). Intimate contacts of the open and unknown sputum group showed almost equal, but a higher percentage of, reactors than the 'closed' group.

Disease—Active lesions were noted in higher percentage (12.1%) among intimate contacts than among distant contacts (4.1%), while arrested lesions and tracheobronchial adenopathy were higher among distant contacts (8.3% & 33.3% respectively against 5.8% and 28.7% in intimate contacts). Thus the incidence of infection and total parenchymal lesions was higher among intimate contacts than among 'distant' contacts.

(c) Continuity of contact.

Infection and disease—The incidence of infection was higher in the 'recent' group (77.5%) than in the 'immediate' group (64.4%), whereas the incidence of demonstrable X-ray evidence of the disease is equally distributed in both the above groups (46.8%).

(d) Duration of contact.

Infection and disease—The percentage of positive reactors and of detectable X-ray lesions increases with the prolongation of the duration of contact with the open source.

Other findings.

The percentage of reactors has been found to be higher among contacts showing parenchymal lesions than among those showing tracheobronchial adenopathy.

The incidence of a combination of active and arrested lesions and of tracheobronchial adenopathy, as well as the different varieties of lesions separately taken, varies *directly* with the intensity of the tuberculin reaction. Thus the probability of finding a tuberculous lesion (esp. parenchymal) in the children becomes more with the stronger reactions.

Twenty percent of the tracheobronchial adenopathy series in the 'open' group has been found to show associated parenchymal lesions (8.5% 'active' and 11.5% 'arrested'). The maximum incidence of this adenopathy occured after 3-4 years' contact with the source of infection and in the 5-10 years age period.

To date, 114 tubercular homes have been surveyed. The findings are identical with those reported last year. No correlation could be found between the incidence of infection and morbidity with economic status or the general health of the contacts. Among the child contacts in these homes tuberculosis mortality was found to be 50% higher than the mortality due to causes other than tuberculosis.

TYPING OF TUBERCLE BACILLI FROM PATHOLOGICAL MATERIALS.

Two hundred and eighteen strains have hitherto been isolated, of which 120 are from glands, pus, urine, skin lesions and other materials from non-pulmonary sources, 37 from tracheobronchial glands and 61 from sputum, lung tissue and pleural fluid. Of these, 212 strains have proved to be of the human type and the remaining six are still under investigation.

It is proposed to include the typing of 50-60 strains from sputum, in this line of investigation, to enable us to declare whether pulmonary tuberculosis in this country arises from bovine infection, as has been found to be the case in some countries.

TUBERCULIN SURVEY OF CERTAIN AREAS INVESTIGATED 10-12 YEARS AGO.

Three hundred and twenty-nine students from two schools and 217 prisoners from one jail have been examined first with V.Pirquet test, as was done in 1927. The results show a definite increase (from 18-27% and from 22-49% respectively) of tuberculous infection in each case—6 to 9% among students and 27% among prisoners. The Pirquet negative cases were also tested with Mantoux test. If the results of this test are added, this increase of tuberculous infection over previous figures becomes 21% among students and 37% among prisoners.

33. Enquiry into the incidence of positive Mantoux reactors and latent tuberculosis in medical students at the King Edward Medical College, Lahore, under Major G. F. Taylor, I.M.S.

The following results were obtained during 1938-39.

MANTOUX EXAMINATIONS.

1938	Total	Mantoux Positive	Mantoux Negative.
1st year Students	62	38 or 61%	24 or 39%
2nd „ „	58	42 or 72%	16 or 28%
3rd „ „	74	56 or 76%	18 or 24%
4th „ „	66	57 or 86%	9 or 14%
5th „ „	59	51 or 86%	8 or 14%
Total	319	244 or 77%	75 or 23%
1939			
1st year Students	82	42 or 51%	40 or 49%
2nd „ „	70	49 or 70%	21 or 30%
3rd „ „	62	43 or 70%	19 or 30%
4th „ „	82	68 or 83%	14 or 17%
5th „ „	43	42 or 98%	1 or 2%
Total	339	244 or 73%	95 or 27%

During this period of two years, 68 students who were negative reactors have become positive reactors. These new positive reactors have been examined clinically, radiologically and their weight, temperature and sedimentation rates measured. None of this group have gone down with clinical tuberculosis.

During this period 18 students who were positive reactors have become negative. This change from the positive to negative reaction has been noted in Europe, and its significance is not yet clear. It shows that the Mantoux skin reaction is not a completely reliable guide to the incidence of tuberculous infection.

A high rate of + + + and + + + + reactions were noted and the variations of these reactions and their significance are being studied.

X-RAY EXAMINATION.

1. All negative reactors who became positive have had skiagrams of their chests taken but none of this group has shown any sign of lung infiltration.

2. The 18 students who showed signs of abnormal skiagrams reported last year have been kept under observation, repeated skiagrams have been taken but none of this group has developed clinical tuberculosis or shown signs of a spreading lesion.

3. The new first year students were examined radiologically. Of 80 students examined, 7 show well marked signs of active or healing

tuberculous lung infiltration. These have been examined clinically and are under observation, the skiagrams of their chests being repeated at 3 or 6 monthly intervals.

Another 12 students of the new first year showed abnormal skiagrams of a doubtful nature and are being kept under observation. The reaming 61 students had normal chest skiagrams.

4. During the period 3 students have developed "clinical" tuberculosis. One died within a few months. He had previously refused to be examined clinically or radiologically. A second developed tuberculosis and is doing well with A.P. treatment. His Mantoux was previously positive and his X-ray taken before the onset of his illness had been passed as normal. On an examination of the earlier skiagram taken before clinical symptoms and signs had developed, the right apex was seen to have a small indefinite shadow of early infiltration. This case illustrates the difficulty in interpreting skiagrams.

The third case developed tuberculous pleurisy with effusion and is now fit again. He had previously refused permission for a cheast skigram and Mantoux reaction to be done.

INDIGENOUS DRUGS.

34. Indigenous drugs enquiry under Brevet Colonel R. N. Chopra, I.M.S. (Ret.) at the School of Tropical Medicine, Calcutta.

INDIGENOUS DRUGS.

(1) *Medical plants* : Chemical investigations of the seeds of *Argemone mexicana* were carried out. The alkaloids obtained from the seeds have been found to be non-toxic, whereas the oil extracted from the seed and free from the alkaloids has proved to be toxic to rats, guinea-pigs and human volunteers. It has been shown by feeding experiments in man that this oil can produce signs and symptoms of epidemic dropsy. The active principles of *Chrysanthemum cinerariifolium* and *C.coccineum* were isolated and their comparative study as regards their insecticidal properties was carried out. These active principles are the two complex esters, pyrethrin I and pyrethrin II. The average pyrethrin content of the flower heads of *C.cinerariifolium* from Kashmir was found to be 0.702 per cent as compared with the figure 0.247 per cent which is obtained from *C.coccineum* from Murree hills. The alkaloid isolated from *Skimmia laureola* was shown to be identical with skimianine found in *S.japonica*. The yield of the pure alkaloid amounts to 0.25 gm. per kg. of powdered leaves. It is insoluble in most of the solvents, being only soluble in dilute mineral acids in excess. The pharmacological action of the essential oil isolated from *Curcuma longifolia* has been worked out. In dilution up to 1 in 100,000 the drug appears to be markedly toxic to paramoecium. A preliminary investigation on the pharmacological action of 'Alanto lactone' obtained from *Inula helinium* shows the drug to be distinctly toxic to earthworms in dilution of 1 in 30,000.

Other drugs under investigation are : (a) *Trianthema Portulacostrum* (*T.monogyna* and *Boerhaavia diffusa*. Chemical analysis of these two drugs has been completed and pharmacological examination of the active principles obtained is being worked out. (b) *Lochnera rosea* (*Vinca rosea*) contains two alkaloids in fair quantities. These are being isolated in pure condition for chemical and pharmacological test. (c) *Cleistanthus collinus* has been found to contain a resin, small quantities of saponins and tannins. It has been stated to be poisonous but no part of the extract obtained by different solvents has been found to be toxic to mice. (d) The chemical composition of *Cassytha filiformis, Sphaeranthus indicus, Tribulus terrestris* and *Phyllanthus urinaria* is in progress.

(2) *Herbarium of medicinal plants* : Work in this connection has been continued. The herbarium now represents about two-third of the total species of known medicinal and poisonous plants in India. It is already becoming known in the country for its authentically named specimens and it will be a pity if this valuable collection, on which work was begun nearly nine years ago, is not completed. As most of the easily available specimens have already been collected and those from inaccessible places remain, the progress has been comparatively slower. It is hoped that in the course of the next two years it will be completed. It has been agreed that of the two complete sets of sheets, one will be left in the Museum of the School of Tropical Medicine and the other sent to the Forest Research Institute, Derha Dun. This collection is unique and this plan will increase the scope of its availability and the chances of perpetual maintenance.

(3) *Poisonous plants of India* : The monograph on "Poisonous plants of India", referred to in the last year's report has been completed and is now ready for the press. This has entailed enormous labour on the part of all the workers of this enquiry. The Imperial Council of Agricultural Research have undertaken to publish this monograph. Nearly three hundred plates drawn by experienced artists have been prepared which are now being reproduced. The completion of this work does not of course mean that everything in this connection has been done. All it means is that important basic work has been completed and all the work so far done in the School of Tropical Medicine and elsewhere has been recorded. Much, however, remains to be done but anyone can now take up investigation of the individual plants without difficulty.

(4) *Snake venoms* : Work on cobra venom was continued chiefly in connection with its therapeutic applications. Cobra venom is undoubtedly of value in relieving the pains in nerve type of leprosy. A combination of Cobra and Russell's Viper venom is now being tried in the treatment of leprosy with encouraging results.

Drug addiction.

This enquiry was closed in 1936 but some important work which required completion is being carried out by the workers of this enquiry. The treatment of opium and morphine habits, which has been introduced by the author, has now established itself and during the year it was tried on a mass scale by the Government of Assam, several thousand cases having been treated. The details of this treatment were published in the Indian Medical Gazette, May 1937, but since then further improvements have been effected in that instead of giving lecithin by the mouth, which sometimes produces nausea and vomiting, it can be given in the form of a specially prepared colloidal solution by the intramuscular route. By this means the quantity of the drug required is considerably reduced and effects are obtained more expeditiously. The rationale of the action of glucose was worked out. It was shown that withdrawal of opium causes an increase in the total proteins in the blood serum, thereby indicating a loss of fluid from the blood. The administration of lecithin and glucose brings the total proteins to their pre-withdrawal level.

Work on hemp drug addiction was completed and a *Memoir* of 119 pages (No. 31) was published in July 1939 embodying the results of about ten years' work in the field and in the laboratory. It has been estimated that there are more than one million persons (approximately 0.5 to 1.9 per cent. of the population) in British India who use hemp drugs habitually. Confirmed ganja and charas smokers, especially those taking large doses, exhibit signs of deterioration of general health, the normal functioning of the central nervous system is impaired, the addict becoming incapable of mental exertion. Temporary and permanent mental derangements, which may lead to violent crime, have been produced.

Chemotherapeutic and Pharmacological investigations.

A compound containing copper and some herbs used in the indigenous systems of medicine in the treatment of malaria, was tested with negative results. Side by side trials of cilional or (certuna) in crescent carriers showed that a total dosage of 0.35 to 0.4 gm. of this drug, administered in doses of 0.03 gm. three times a day, is usually sufficient to

eradicate gametocytes of *P.falciparum* from the blood and even this high dosage has been borne by patients without untoward symptoms. Plasmochin would, however, be preferable to cilional, inasmuch as a much smaller dose of the former effects the eradication of crescents in a comparatively short time, and such dosage of plasmochin is not at all toxic and is cheaper than cilional.

It was shown in simian malaria that both prontosil and soluseptasine have a definite action on *Plasmodium knowlessi* in dosage which is even less than what is regarded as the proportionate dose for a monkey as compared with that of man. Trials of prontosil in ordinary therapeutic doses of 3 to 4 gm. daily for four days undoubtedly caused the disappearance of malarial parasites from the peripheral blood and controlled the symptoms of the disease. It destroys both the asexual and sexual forms of *P.vivax* and *P.malariae* but only the sexual forms of *P.falciparum*. It has no action on crescents. Its action on *P.malariae* is comparatively slower and less potent. The drug is worthy of trial when other antimalarial drugs are not available.

Atebrin was found to be an effective drug in eradicating infection with *Giardia lamblia*. Administration of nine tablets as a maximum brought about the disappearance of the parasite from the stools in every case. It has no such action on other flagellates of the intestines occuring in India, *e.g.*, *Chilomastix*, *Trichomonas* and *Enteromonas*.

As a result of investigations with a large number of drugs in the treatment of filarial infection it was found that, of the organo-metallic compounds, soamin appears to be the most satisfactory in controlling infection in early stages. No drug has been found so far that has satisfactory antifilarial properties.

35. Indigenous drugs enquiry under Dr. Khem Singh Grewal at the King Edward Medical College, Lahore.

Hiran Tutia.

An indigenous drug sold at Amritsar under the name of *Hiran tutia* was investigated. This drug is used by certain pharmaceutical firms in India as a substitute for senega for the preparation of official preparations of senega. It has been found to contain a saponin to the extent of 1 per cent. The saponin is soluble in water and alcohol. It is insoluble in organic solvents other than alcohol. The purification of the saponin has been dried by dialysis, by the copper method and the baryta method. The saponin is being collected in a purified state for further investigation.

An attempt has been made to find out the botanical source of the drug. A sample of the drug containing leaves, seeds and roots was procured. It was identified by the Punjab University Botany Department as *Euphorbia thomsoniona*. As the sample contained no flowers, a fresh sample containing flowers is being procured.

Thalectrum Javanicum.

The plant from Solon was investigated last year and was shown to contain berberine. A sample of the rhizome was procured through the utilization Department of Kashmir State. This sample contained only 0.4 per cent of berberine, a smaller quantity than that contained in the sample from Solon. The Kashmir rhizome was about fifteen years old whereas the rhizome from Solon was about three years of age. Berberine contents of rhizomes at various ages from Solon and from Kashmir are being investigated.

Brahmi.

Brahmi which is a well known drug in the Punjab is being investigated. A preliminary examination showed it to contain glucosides. Twenty gms. of the plant were extracted in hot alcohol of sp. gr. 0.848. The extract was concentrated under vacuum. The dried extract weighed 3.987 gms. It was dissolved in 200 c.c. of alcohol sp. gr. 0.848 and divided in aliquot parts.

The glucoside was extracted with hot alcohol in the presence of calcium carbonate. Alcohol was evaporated under vacuum. The extract was nearly dried and extracted with distilled water. The water soluble extractive is 3.2 per cent. The water was removed under vacuum. The residue was taken up with absolute alcohol. The glucoside from absolute alcohol solution was obtained by fractional precipitation with ether. Further purification of these fractions is being attempted.

Pyrethrum flowers.

First year flowers of pyrethrum from Kashmir were examined for pyrethrum content by the method given in B. P. C. The sample contained 0.86 per cent of pyrethrin 1. Toxicity of the alcoholic extract dissolved in kerosine oil was determined on mosquitoes and flies. The sample has been found to be very toxic both to flies and mosquitoes.

Rhamnus Dahaurius.

The fruits and the bark of this plant are under investigation. The fruit is used as a purgative in Kashmir.

Rasaut.

Investigation on rasaut is being carried out. The toxic substance, besides berberine contained in rasaut, is evading isolation. Attempts are being made to procure it in a purified state.

Nelumbium

Nelumbium alkaloid was further purified. This investigation was being done with the help of Professor Barger of Glasgow, whose sudden and unexpected death on January 5, 1939, has resulted in a setback in the study of this problem. The help of Professor Burn of Oxford University is being sought. Micro-chemical analysis is not possible in Lahore. The late Professor Barger got a micro-chemical analysis done by Dr. Rath of Hadlaberg on the alkaloid that was sent to him.

36. Investigation of indigenous drugs under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay.

The grant was available from the 1st of April, 1939 and the work began with the chemical analysis of *Solanum Xanthocarpum* (vern. Bhoiringni). The drug has been recommended as an antipyretic, diaphoretic and diuretic and is a remedy recommended for heart disease.

It was analysed chemically and (1) an oil, (2) a solid m.p. 248°, probably a phytosterol, (3) a glucoalkaloid, m.p. 283°, yielding on hydrolysis an alkaloid, m.p. 198°, were isolated.

The *glucoalkaloid*, m.p. 283°, is sparingly soluble in pure water but is soluble in acidulated water of pH 6.

The *alkaloid*, m.p. 198°, is insoluble in water. Further the salts of the alkaloid (hydrochloride and sulphate) were prepared in view of the fact that salts of alkaloids are more soluble in water than the alkaloids themselves, but were found to be insoluble in distilled water as well as in acidulated water of pH 6.

The *tincture* of the drug, after defatting it with petroleum ether, is prepared by extracting with alcohol and 1 c.c. of this tincture is equivalent to 4.13 mgm. of the glucoalkaloid. It was also used in our experiments.

Firstly, *toxicity* tests were made on rats. The solution containing 4 mgm. glucoalkaloid was administered by mouth and hypodermically to rats weighing between 119 to 123 gms. but no noticeable effects were observed on them. Similarly no action was noticed on frogs weighing between 98 to 120 gms. with the same dose. Further experiments were performed with larger doses of the tincture of the drug and of the glucoalkaloid on frogs. An approximate dose of 4 c.c. of the tincture (containing approximately 16.5 mgm. of glucoalkaloid) per 100 gms. of frog was used in a number of frogs. It was observed that all the frogs were paralysed within 10 minutes to 1½ hours after the injection. Their pupils were contracted. The paralysis was not preceded by any convulsions or spasms. It was found that absorption of the substance from the ventral lymph sac was much quicker than from the leg. Some more frogs were injected subcutaneously with 16 mgm. of glucoalkaloid per 100 gm. of frog. Frogs receiving injection in the ventral lymph sac were found to be paralysed within half an hour to two and a half hours. However the majority of those receiving injection in the leg were found dead after 24 hours.

Sciatic nerves of frogs which were paralysed after an injection of the tincture in the leg were stimulated and it was found that a stronger current was required to elicit a contraction on the injected side than on the uninjected one. Further experiments to determine the action of the drug on peripheral nerves and muscle are in progress.

After an intravenous injection of the solution of the glucoalkaloid or the tincture, a slow, continuous, prolonged rise of blood pressure was observed in dogs under urethane anesthesia. After the injection of the tincture there was a transitory fall before the rise commenced. This preliminary fall of blood pressure was less when a dialysed tincture was used and was absent after the glucoalkaloid. When the glucoalkaloid was

perfused through the blood *vessels* of the frog the rate of flow through the vessels diminished. It produced an increased tendency to systole with incomplete diastole when it was perfused through isolated frog's heart and with higher concentrations there was systolic arrest of the heart. There was no change in the rate. When it was circulated through the frog's heart in situ, this systolic tendency was observed, but there was a peculiar action on the auricles which swelled up and assumed a jelly-like appearance in a few minutes, ultimately, the ventricles got embedded in this jelly and stopped beating. The nature of this phenomenon is being studied.

MATERNAL MORTALITY.

37. Enquiry into anaemia of women under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta.

SUMMARY OF THE DISCUSSION ON THE WORK.

During last year Dr. Majumdar's main work has been on experiments with monkeys. The object of these experiments has been to subject these animals to the conditions that appear to produce anaemia in patients, to follow the blood changes by frequent, sometimes daily, examinations, and the tissue responses, by biopsy and autopsy, and eventually to test the response to treatment. The methods adopted have included repeated small bleedings (the parallel to hookworm infections), malarial infections, and restrictions of dietary. So far these experiments have done little to throw any light on the problem. They have however produced useful basic data, including for example 115 total blood counts on 33 normal monkeys on which a paper is being written, and have shown the effect of repeated small bleedings and of acute malaria. No condition comparable to chronic malaria has been produced.

A new series of experiments has been commenced in which it is proposed to try certain biochemical methods of affecting the blood picture.

Tropical macrocytic anaemia is being studied in the hospital here, where always about 10 cases are undergoing experimental treatment with different liver fractions and Dr. Majumder has helped with these examinations from time to time. He has also helped in the examination of another series of normal school children to which he refers in his report.

Dr. Majumder is now working in Shillong, investigating the anaemia of pregnancy which is very prevalent there and also obtaining normal data from the local population, after a preliminary survey had been carried out by Dr. C. R. Das Gupta who went to Shillong for this purpose on Dr. Napier's behalf in October.

A short report of Dr. Majumder's work prepared by himself is appended.

APPENDIX.

The work done under this enquiry may be broadly classified into two groups: (A) experimental (B) clinical.

EXPERIMENTAL WORK.

(I) Last year, it was shown by repeated bleedings, that definite microcytic hypochromic anaemia could be produced in monkeys. Feeding with deficient diet, devised by Lucy Wills, however, did not produce typical macrocytic anaemia and was, therefore, continued. Throughout the year, 5 monkeys had been kept on this diet and the period through which these animals were on this diet varied in individual cases from 8 to 14 months; of these 4 have already died and one is still surviving. Blood pictures before and after the experimental period are shown below against each of these monkeys.

		Hb.	R. B. C.	R' cyte.	C. V.	M. C. V.	M. C. H.	M. C. H. C.
1. Monkey No. 7	Before	14.30	5.94	3.2	48.0	80.8	24.0	29.8
	After	11.96	5.35	2.0	44.3	82.8	22.3	26.9
2. Monkey No. 8	Before	12.1	5.46	0.2	46.0	84.2	22.2	26.3
	After	10.18	4.56	1.4	36.5	80.0	22.3	27.9
3. Monkey No. 12	Before	12.92	5.64	1.0	44.5	78.9	22.9	29.1
	After	11.96	4.63	5.2	43.1	93.0	25.8	27.7
4. Monkey No. 13	Before	13.06	5.75	2.0	43.0	74.7	22.7	30.3
	After	12.1	4.16	1.0	43.0	103.3	29.1	28.1
5. Monkey No. 14	Before	13.06	5.48	1.2	47.1	85.9	23.8	27.7
	After	6.33	2.83	2.0	23.3	82.2	22.3	27.1

It is found that in almost all these cases there is distinct anaemia as shown by a definite fall in the level of haemoglobin and R. B. C., but the evidence is not sufficient to show that there is any tendency towards macrocytosis; nor is it adequate to disprove any such tendency.

(II) A new diet of rice, casein and a special salt mixture of calcium, magnesium, potassium etc., was tried on 3 monkeys in an attempt to produce anaemia artificially. The animals were on this diet for about 5 months. The blood changes produced by this diet are shown below :—

		Hb.	R. B. C.	R' cyte	C. V.	M. C. V.	M. C. H.	M. C. H. C.	W. B. C
1. Monkey No. 17	Before	14.30	5.82	2.3	44.5	76.4	24.6	32.1	21.7
	After	10.45	3.91	1.3	41.0	104.8	26.7	25.5	23.9
2. Monkey No. 18	Before	11.96	5.20	0.6	43.0	82.7	23.0	27.8	22.4
	After	11.96	4.71	0.7	39.5	83.8	25.4	30.7	10.0
3. Monkey No. 20	Before	13.75	5.39	0.6	46.0	85.5	25.5	29.9	28.3
	After	12.38	4.90	1.0	41.7	85.1	25.3	29.7	12.1

The results, here, are almost indistinguishable from those obtained with 'Lucy Wills' diet except that with this diet, the average survival period of the animals seemed to be less than with Lucy Wills' diet.

(III) Production of chronic anaemia with malarial infections was tried in 3 monkeys. In 2 cases, it was not successful since the animals developed sudden acute anaemia and succumbed to the effects thereof. The third monkey was successfully infected repeatedly for 5 times with either *Plasmodium knowlesii* or *P.inni*. It was found that each time this particular monkey recovered from anaemia, it required an increasing dose of malarial infection to induce further anaemia. The nature of the anaemia produced was generally normocytic.

(IV) Lately, a new series of 4 monkeys has been taken, and it is intended to perform with these some fresh experiments designed to tackle the aetiological problems of anaemia from a new angle.

Since January, in all 283 complete blood examinations have been done on these experimental monkeys.

CLINICAL WORK.

(1) Although no particular series of patients was investigated with especial reference to this enquiry, a large number of blood, marrow and reticulocyte counts was done almost daily in connexion with special cases under investigation either as indoor or outdoor patients of the hospital.

(2) In connexion with the question of variation of blood picture with differences in the diet, an investigation was made into the normal haematological standards of Punjabi school children in a High School. This enquiry to which assistance was given, comprised physical examination and haemoglobin determination in 282 school children of both sexes of different ages varying from 5 years to 15 years.

38. Enquiry into the toxaemias of pregnancy under Dr. N. A. Purandare, at the Seth G. S. Medical College, Bombay.

This year, investigations were continued with a view to finding out the morbid changes produced in different organs in cases of pre-eclampsia, a condition in which it is very difficult to obtain post-mortem material. It was therefore decided to study the histological changes in the viscera of pregnant guinea-pigs after a long course of injections of placental extract.

Fifty-two histological sections were first prepared from various organs of normal non-pregnant and pregnant guinea-pigs to serve as controls for this study.

Experiments were then carried out to decide a suitable method of preparing the placental extract: the following types of placenta were used :—

(*a*) placentae from normal pregnant patients.
(*b*) placentae from pre-eclamptic cases.
(*c*) placenta from an eclamptic case.

Five hundred and seventy microscopic slides were prepared in carrying out 23 experiments in all. The methods used in preparing the extracts were as follows :—

(1) simple saline extract—both autolysed and non-autolysed —(Bartholomew's method).
(2) extracts of acetone fixed placentae.
(3) extract of dried placentae. (whole).
(4) saline extracts further fractionated into albumin fraction as well as globulin fraction.

DETAILS OF THE EXPERIMENTS.

1. *Experiments with saline extract*—(Bartholomew's method).
Normal healthy placentae.

(*a*) autolysed extract—injected into 9 pregnant guinea-pigs, subcutaneously and in one intracardiac.

(*b*) non-autolysed extract—injected into 8 pregnant guinea-pigs subcutaneously and in one intracardiac.

Pre-eclamptic placentae.

(*a*) autolysed extract—injected subcutaneously into pregnant guinea-pigs.

(*b*) non-autolysed extract—injected into 7 guinea-pigs subcutaneously.

Eclamptic placentae.

(*a*) autolysed extract—injected into 3 pregnant guinea-pigs subcutaneously.

(*b*) non-autolysed extract—injected into 3 pregnant guinea-pigs.

2. *Experiments with extracts of acetone-fixed placentae* :—

(*a*) normal healthy placentae—injected into 3 pregnant guinea-pigs.

(*b*) pre-eclamptic placentae—injected into 6 animals.

(1) three with filtered extract.

(2) three with non-filtered extract.

3. *Experiments with extracts of two dried pre-eclamptic placentae (whole)* :—

The total number of animals injected with these extracts was eight.

As the last two methods did not show any material advantage over the previous experiments, it was decided in consultation with Dr. Dikshit and Professor Khanolkar, to use the Bartholomew's method of saline extraction. Further the autolysed and non-autolysed extracts thus prepared were fractionated with the help of Dr. Dikshit in the Haffkine Institute into two parts—albumin fraction and globulin fraction. They are at present used for injection into animals, so as to decide which of these portions of saline extract was responsible for the changes so far obtained, after the injections of the whole saline extract.

4. *Experiments with injections of albumin and globulin fractions of normal placentae from autolysed & non-autolysed saline extracts* :—

(*a*) Normal placentae :—

autolysed part—albumin fraction injected into 4 animals and globulin fraction into one animal.

non-autolysed part—albumin and globulin fractions injected into one animal each.

(*b*) Pre-eclamptic placentae :—

autolysed part—albumin fraction injected into 2 animals and globulin fraction into 2 animals. Besides two more animals are being injected.

RESULT OF HISTOLOGICAL EXAMINATIONS (IN BRIEF).

The histological sections were prepared from liver, kidney, spleen, suprarenals, brain, ovaries and fallopain tubes. The findings from the liver, kidney and suprarenal sections were more obvious and were as follows :—

Autolysed saline extract from normal placenta—

(*i*) subcutaneous injections :—

Liver.—Some degree of congestion of the liver with dilated portal veins. The intracellular sinusoids are also dilated and filled with R. B. C especially when a prolonged course of injections was given.
(2) Blue stained cells around the portal vein which are normally seen, are much more conspicuous.
(3) The central hepatic cells show vacuolation in the cytoplasm yet the individual cell outlines are distinct.
(4) There are no areas of complete degeneration or necrosis of the liver cells, though the latter show coagulative changes especially in the periportal region.

Suprarenal.—(1) Disappearance of the intracellular vacuoles of the cortical cells.
(2) Occasional areas of haemorrhage.
(3) Deposit of yellow pigment in the deeper part of the cortex.
(4) Slight congestion of the medulla but no other change in it.

Kidney.—(1) The size of the glomerular tuft is increased and it practically fills up the whole of the capsule. The capillaries of the tufts are dilated and filled with R. B. C.
(2) The cells of the convoluted tubules are degenerative with the cell outlines indistinct and the nuclei badly stained.
(3) In the cells of the collecting tubules the degenerative changes are not clear.
(4) There are but few areas of haemorrhage. But in experiments with the *non-autolysed extract* no such changes are noted.

(*ii*) Intracardiac injections :—

The above mentioned changes are much more conspicuous.

Liver.—(1) Definite columns of degenerated and deeply stained hepatic cells in the periportal region.
(2) Areas of liquefaction and necrosis also present in these areas.
(3) Haemorrhages in the liver tissue with dilated sinuses.

(4) The nuclei of the hepatic cells around the central veins well stained and the cytoplasm shows plenty of vacuoles.
(5) All these changes are diffuse.

Suprarenals.—(1) Very few intracellular vacuoles.
(2) Zone glomerulosa slightly increased in extent.
(3) Areas of haemorrhages in the zone fasciculata, the cell outline in which zone is often indistinct.
(4) Few yellow pigmented cells in zona veticularis.

Kidney—(1) The glomeruli are swollen with increased cellularity of the tuft.
(2) No congestion.
(3) The capsule is normal.
(4) The convoluted tubules have their lumena filled with coagulated material with complete disappearance of the cell nuclei at some places.

Non-autolysed saline extract from normal cases :—

Liver.—The above changes practically absent.

Kidney.—The above changes found practically absent.

Autolysed saline extract from pre-elcamptic cases—in addition to the above changes—

Liver.—(1) Areas of haemorrhages.
(2) Marked pigmentation in the cells of sinusoid.
(3) Big areas of infarcts with polymorph infiltration at their borders.
(3) The nuclei of hepatic cells in these areas completely broken down.

Saline extract from eclamptic cases :—

These experiments will have to be repeated before definite conclusions can be drawn.

Extract of Acetone fixed placenta :—

No additional changes.

Dried placental extract :—

Liver.—(1) Yellowish pigmentation in Kupffer's cells.

Kidney.—(1) The swollen glomeruli fill up the capsules.
(2) The lining epithelium of the capsule is not affected nor are there adhesions between the tufts and capsule.
(3) The organ is congested with occasional haemorrhages.
(4) The convoluted tubules in these areas are necrotic or showing marked degeneration.
(5) No haemorrhages are seen inside the capsule or the lumen of the convoluted tubules.
(6) Pigment is also present in the cells of the tubules especially the collecting ones.
(7) The latter are very little altered except for marked congestion and haemorrhages around them.

(The changes are seen all over the organ and not localised to one part.)

As the last two methods of preparing the placental extract did not show any material advantage over the previous method used, it was decided to use again saline extract for injection after autolysis. But the extract was further separated into two fractions 'albumin fraction' and 'globulin fraction' by half and full saturation methods.

(*a*) *Injection of albumin fraction* :—(from a normal placenta).

Liver.—(1) Slight hyperaemia.
(2) Fatty degeneration in the hepatic cells—specially seen in the area around the central veins.
(3) Albuminoid changes in the cell sytoplasm.
(4) Staining of the cell—nuclei normal.
(5) No infiltration by polymorphs.

Kidney.—(1) Slight degree of congestion of the glomerular tuft which is otherwise normal.
(2) Convoluted tubular epithelium shows varying degrees of degeneration.
(3) Collecting tubules unaffected.

Suprarenals.—No changes.

(*b*) *Injection of globulin fraction* :—

Liver.—(1) Congested organ.
(2) Fatty degeneration—only to a very slight degree and diffused.

Kidney.—(1) Increased cellularity of the tuft, outline of which is irregular ; engorgement of the tuft, capsular spaces obliterated.
(2) Convoluted tubules show degenerative changes.
(3) Minute haemorrhages in the medulla.

Suprarenal.—No changes.

Histological sections.

Histological sections of the normal and morbid placentae, used for extraction in the experiments, were prepared. In addition, sections were also taken from a placenta of 3 months and one from a case of chronic nephritis complicating pregnancy for comprision. So far the sections collected are few yet some distinct findings could be gathered :—

(1) The lining syncetium of the villi shows distinct changes of degeneration in the large percentage of the villi.

(2) There is very little change observed in the blood vessels, embryonic connective tissue of the villi of pre-eclamptic placenta as contrasted to that of chronic nephritis which however shows a normal lining of syncetium.

Bio-chemical examination of blood and chemical examination of urine.

These were repeatedly carried out to confirm the diagnosis of eclampsia and pre-eclampsia before making use of the placentae for experiments.

Sixty-four samples of blood were examined for detailed bio-chemical investigations. They were obtained from 33 cases of pre-eclampsia. In some cases blood was examined repeatedly before and after delivery.

Pre-eclampsia.	*Eclampsia.*
NON-PROTEIN NITROGEN.	
Normal—27 cases.	Normal—11 cases.
Increased—10 cases.	Increased—6 cases.
Decreased—Nil.	Decreased—Nil.
UREA NITROGEN.	
Normal—21 cases.	Normal—11 cases.
Increased—6 cases.	Increased—4 cases.
Decreased—13 cases.	Decreased—4 cases.
URIC ACID.	
Normal—13 cases.	Normal—2 cases.
Increased—29 cases	Increased—17 cases.
Decreased—Nil.	Decreased—Nil.
SERUM CALCIUM.	
Normal—8 cases.	Normal—5 cases.
Increased—Nil.	Increased—Nil.
Decreased—3 cases (Slightly).	Decreased—2 cases (Slightly).
CREATININE.	
Normal—10 cases.	Normal—7 cases.
Increased—Nil.	Increased—Nil.
Decreased—Nil.	Decreased—Nil.
INORGANIC PHOSPHATES.	
Normal—10 cases	Normal—7 cases.
Increased—Nil.	Increased—Nil.
Decreased—Nil.	Decreased—Nil.

Average values :—

(From 80 blood samples)

	N.P.N.	Urea N.	U.N./ N.P.N.	Uric acid	Uric acid/ N.P.N.
Normal pregnant woman	20-35	10-15	0.28 to 0.6	2 to 3	10%
Pre-eclampsia (60 cases)	30.8 mgm.	9 mgm.	0.3	3.7 mgm.	12%
Eclampsia (20 cases)	32.4 mgm.	12.8 mgm.	0.4	4.6 mgm.	14%

Uric acid :—Among the pre-eclamptic patients, the largest number of cases showed the amount of uric acid in the region of 3-4% whereas in the eclamptic patients the uric acid values were all above 4 mg. per cent;

the maximum number of cases was found to be between 4 to 6 mg. per cent. The highest figure obtained in one case was 7.3 mgm. Uric acid value is of not so much significance as the ratio between uric acid and N. P. N.

BIOLOGICAL EXAMINATIONS.

Estimation of A. P. L. in :—

(*a*) the blood in 7 cases of pre-eclampsia and 3 of eclampsia.
(*b*) urine—In 5 cases of pre-eclampsia.

Estimation of oestrin in the urine of a healthy pregnant woman did not reveal any constant and conclusive findings.

Massive doses of A. P. L. (Antuitrin 'S') were injected into 2 guinea-pigs to find out the changes produced in the organs. But only the ovaries showed changes, the rest of the organs did not show the degenerative changes as seen in the animal experiments after injecting the placental extract. Injections of A. P. L. were done as follows :—

Animal No.—(1)
19 injections of 1 c.c. A. P. L.
1 injection of 0.4 c.c. ,,

Animal No.—(2)
9 injections of 2 c.c. A. P. L.
1 injection of 0.4 c.c. ,,

39. The Delhi maternal mortality enquiry under Dr. M. I. Neal Edwards, W. M. S.

This enquiry is being conducted on the same lines as the earlier maternal mortality enquiries in Calcutta and Bombay.

AREA.

The area chosen is Delhi city, New Delhi and the Notified Areas with an urban population of 428,000 and Najafgarh Health Unit with a rural population of 42,000. The total births registered in the urban area during 1937 were 20,540 and in the rural area 1,193.

METHOD OF WORK.

An arrangement was made with the Chief Health Officer, Delhi Province, whereby we receive intimation of every female death between the ages of 10 and 50 years. The Chief Health Officer very kindly arranged further that, in the case of each female death during this age period, the question of association with pregnancy or childbearing should be ascertained. This information is being collected by the Municipal sanitary officers appointed to follow up deaths in Delhi city, by health visitors in New Delhi and in Najafgarh, and by the superintendents of hospitals in the case of hospital deaths. We are grateful to these officers for their

help, without which the work would have been impossible. All female deaths which occurred in pregnancy, labour, or within 3 months of delivery, are then followed up and investigated in deatil by Dr. J. Dei, M.B., B.S., who was appointed Research Assistant. The follow-up of deaths was started in March 1939 and has been carried on continuously for 7 months. The enquiry will be completed by the end of March 1940.

Summary of findings.

During the period of 7 months 207 maternal deaths have been investigated of which 190 were registered in the urban area and 17 in the Najafgarh area. In addition to the deaths occurring in the Najafgarh (rural) area, there were, however, some imported cases which belonged to other rural districts, but died in Delhi, so that their deaths were registered in the urban areas. The total number of deaths so far investigated of women living under *rural* conditions is 46 of which 25 were due directly to childbearing and 21 to diseases associated with childbearing. This enquiry is therefore providing a certain amount of information regarding rural maternal mortality.

Amongst the 207 maternal deaths investigated, as many as 122 were of women who were admitted to a hospital in Delhi at some time during pregnancy, labour or the puerperium. In these cases and among those treated by private doctors, a considerable amount of accurate information has been obtainable. In the case of the remaining, the conclusion regarding the cause of death has had to be based only on the history given by relatives or the attendant at delivery, health visitor, nurse or dai. A considerable proportion of the rural deaths fall into the latter category and it has sometimes been impossible to reach a diagnosis, although it has nearly always been possible to decide whether the death was due to a condition due directly to childbearing or to a disease associated with childbearing.

The distribution by cause of 207 deaths so far investigated is shown below both for rural and urban cases and for the total.

Maternal deaths by cause.

Maternal deaths	Urban		Rural		Unknown		Total	
Directly due to childbearing	*Number*	*% of total*	*Number*	*% of total*	*Number*	*% of total*	*Number*	*% of total*
Puerperal sepsis	30	37.6	13	52	5	25	48	37.8
Haemorrhage (A. P. H. & P. P. H.)	16	19.5	1	4	2	10	19	14.9
Accidents of labour	6	7.3			3	15	9	7.1
Toxaemias of pregnancy	4	4.8	2	8	3	15	9	7.1
Abortions & accidents of pregnancy	4	4.8	2	8	3	15	9	7.1
Thrombosis & embolism	5	6.0					5	4.0
Anaemia	17	20.0	7	28	4	20	28	22.0
Total ..	82	100.0	25	100.0	20	100.0	127	100.0

Maternal deaths	Urban	Rural	Unknown	Total
Diseases associated with childbearing				
Tuberculosis	20	5	3	28
Others	33	16	3	52
Total maternal deaths	135	46	26	207

The table shows that, as in the previous enquiries, puerperal sepsis is the outstanding cause and was responsible for 37.8% of deaths due *directly* to childbearing, and as high a figure as 52% of rural deaths.

Anaemia comes next in importance (22%) and the contribution to maternal mortality which can be traced to this condition is by no means confined to the figure shown for deaths from this cause, for a considerable proportion of the women who died from other childbearing causes, particularly sepsis and haemorrhage, were severely anaemic in pregnancy and it is probable that their anaemia was the deciding factor in causing death.

Unlike the findings in Calcutta and Bombay, haemorrhage comes next (14.9%) in order of frequency.

The toxaemias of pregnancy (7.1%) are relatively less important in Delhi than was found in the cities of Calcutta and Bombay.

Tuberculosis stands out as by far the most important cause of death due to conditions *associated with* pregnancy. The same condition was found in Calcutta and Bombay. Tuberculosis appears, from the figures so far available in Delhi, to be considerably more important in women living in urban than in rural conditions.

40. Investigation of biochemical and dietetic factors concerned in the toxaemias of pregnancy under Dr. Vishwa Nath at the King Edward Medical College, Lahore.

Seventy-five cases showing albuminuria during the last trimester of pregnancy and twenty-five pregnant controls were studied in the course of this inquiry. A detailed biochemical investigation of the blood and urine of these cases was conducted and of the diet consumed with regard to quantity and quality. The main findings in the toxaemic group were hypoproteinaemia and hypocalcaemia. The urine showed poor excretion or absence of vitamin C. Variations in the level of these findings as well as other items of investigation were found amongst the different clinical groups composing the toxaemic cases, but significantly subnormal figures with regard to blood calcium and blood porteins and haemoglobin were common to almost all the groups. The diet of these cases was deficient in "protective foods", particularly in animal proteins, minerals like calcium and the vitamins.

MISCELLANEOUS.

41. Helminthological enquiry under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta.

The examination of the worm parasites collected from 100 pariah dogs in Calcutta in the search for *Taenia ovis*, a possible source of *Cysticercus cellulosae* in man, proved unsuccessful, but other facts of interest to human health questions have emerged from this inquiry.

The incidence of *Echinococcus granulosus*, the source of hydatid in man and animals proved unusually high for city dogs whose opportunities of gaining access to infected meat are slight. Seven animals were found to harbour *Trichostrongylus colubriformis*, which is a common parasite of ruminants and is occasionally found in man, particularly in certain districts in India. This is the first record in the world, as far as can be ascertained, of the worm being found in dogs and offers a more likely explanation of the source of infection of human beings than when it was only known to infect domestic stock, which are rarely in such close contact with man as are dogs. A complete report on all the worms collected is almost ready for publication, and in addition to the above findings of human interest, the report should prove of value to parasitologists with a wider interest than that of human infections only.

An attempt was made to find the incidence of infection and districts of origin of pigs killed in Calcutta which were infected with *Cysticercus cellulosae* with the idea of later using the knowledge so acquired in field investigations of the places from which the infected pigs came, because one of the chief difficulties in dealing with this important subject is that the knowledge of the human reservoirs of this infection in India is almost non-existent. Unfortunately this work had to be abandoned on account of the opposition of the commercial interests concerned for fear that our reports might entail financial loss to them.

The qualified assistant attached to this inquiry resigned to take a better post early in the year, and as it was not possible to find one already trained in helminthology to take his place, it was necessary for the new man to spend some time acquiring sufficient knowledge to carry on research work. He has now been sufficiently trained and in November proceeded to an area in Assam to commence a field study of human fluke infections, which from the meagre reports available, make it appear they are much more prevalent in certain parts of the Bramaputra valley than our present knowledge would lead us to suppose. The present tour, which is still in progress, is in the nature of a preliminary survey, and it is proposed to amplify the field inquiry with investigations on the unknown snail hosts etc., in the laboratory, when this worker returns with the necessary material.

42. Medical mycology enquiry under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta.

The determination of the lowest effective dilution of the group of fungicides, which has been going on for some time, has been completed, and the final results will be submitted for publication shortly. In the meantime a paper on the clinical application of these results has been published in the *Indian Medical Gazette*.

A great deal more work has been done on cheiropompholyx and during its progress a remarkable case of generalised moniliasis was discovered. It was fully worked out and cured, and a case note on it was published in the *Indian Medical Gazette*. In consequence of finding this case, a search for similar ones has been maintained and already 15 others have been found. This indicates that monilial dermatitis is much more frequent than has hitherto been recognised, and on account of its apparent importance, this subject is being actively followed up, both clinically and in the laboratory, to find if definitely pathogenic and non-pathogenic varieties of monilia can be distinguished. Also, a case of epidermophytosis in a baby only a few months old was seen and the causal organism was shown to be *Epidermophyton cruris*. This is of great importance because all authorities hold that this fungus never attacks children, and that all such cases in the young are caused by a trichophyton.

The work on virus infections of the skin was brought to a close with the publication of a paper demonstrating a complement fixation reaction in cured cases. Although all attempts to demonstrate viruses, either directly or indirectly, in the other diseases in this group were unsuccessful, the clinical results following the use of specific filtrates from skin lesions have been consistently good, and they have been continued throughout the year.

The complement fixation reaction in a group of various types of skin tuberculosis was carried out and the results were published.

With the discontinuance of the virus work, the treatment of leucoderma by the intracutaneous injection of certain substances into the depigmented spots has been taken up, and the preliminary results have been very good, but as there are many details in this form of treatment to be worked out before a finished report on the subject can be issued, no publication on it has yet been submitted.

43. Dracontiasis and filariasis enquiry under Dr. S. Sundar Rao at the Calcutta School of Tropical Medicine.

Dracontiasis.

A field station for epidemiological studies of guinea-worm infection was established in Vizianagram during the year. A number of endemic

villages near this town has been carefully surveyed. The average population is about 500 in a village, the largest being about 5,000 in the village Kumili. In all the villages there are separate ponds or wells for the supply of drinking water for the different communities. The average rainfall in these villages is below 25 inches per year which appears to be an important factor of endemicity. Seasonal variation of the infection has been observed as in other endemic areas, the majority of the cases occurring during the months of June and July. These are the hottest months of the year in these villages and the supply of drinking water is very scarce at this period. A survey of these villages indicates that the incidence of infection varies from year to year, the incidence being particularly low during the present year. It is interesting to note that a few of the villages are practically free from the infection although they are surrounded by other highly endemic villages. Tunivalsa may be cited as a typical example. This village which was endemic for several years has been free from the infection after the recent conversion of the step well into a draw well which is the chief source of drinking water supply.

A number of adult worms were collected for examination and for the preparation of antigen. Cyclops were also collected from both endemic and non-endemic villages in this area and were identified. Collections of blood from guinea-worm patients were made for complement-fixation tests. Blister fluids were also collected for allergic tests. Experimental infection of cyclops with guinea-worm larvae was carried out at the field station.

Filariasis.

A toxin has been isolated from the hydrocele fluid of filarial patients and has been found to be specific by precipitin and complement fixation tests. Systematic experiments on dermal tests with this toxin have been carried out in a number of cases showing different stages of filarial infection. Tests with control cases have also been carried out side by side. The results obtained with this toxin are promising and indicate that it is likely to be of considerable help in the diagnosis and treatment of filarial infection and disease. It may be noted that a specific antigen is available for the first time, as the antigens used for carrying out these tests hitherto have been only of non-specific types such as extracts of *Dirofilaria immitis* etc. A total of 86 hydrocele fluids has been examined so far. The results show that the amount of toxin varied from fluid to fluid. Further experiments are in progress in collaboration with Dr. R. K. Goyal.

Experiments have been carried out to find out the distribution of toxin antigen in the various albumen fractions of hydrocele fluid in filarial patients.

Mature larvae (W. bancrofti) die instantaneously when treateed with gastric juice and live for about five hours in the solution prepared to resemble the human sweat, (Ammonia, 18 mgm per cent, Urea N, 29 mgm per cent, NaCl 0.4 per cent).

Cultures were made from tissue fluids obtained by puncturing elephantiasis limbs and glands etc. In a total of 50 afebrile cases examined, the cultures were negative showing the absence of secondary infection. In five cases of filarial abscesses the pus showed cultures of streptococci or staphylococci or both.

In view of the increasing importance of vitamin C in acute infections quantitative estimations of this vitamin in the blood of filarial patients were made. No appreciable variation from normal was noticed. The cholenastrase content of the blood and hydrocele fluid of filarial patients was also found to be within the range of normal persons.

Experiments on sternal punctures of filarial patients after local anaesthesia were continued. None of the cases showed anaemia which could be attributed to filarial infection.

Investigations on the quantitative distribution of cholestrin and lecithin in the blood and lymph of filarial patients show that cholestrin is higher than normal while the lecithin content was definitely lower than normal

Adult parasites of Microfilaria malayi.

During November field work was carried out in North Travancore with the main object of isolating the adult of *Microfilaria malayi*. After a preliminary examination of blood for filarial infection in Shertalai (North Travancore) a large number of patients (over 3,500) was carefully examined for filarial cysts. One of them showed a cyst on the forearm. The blood of this patient was examined and was found to show *Microfilaria malayi*. On aspirating the cyst four adult worms were recovered. The lymph from the cyst showed a large number of *Microfilaria malayi*. Neither the blood nor the lymph showed any trace of *Microfilaria bancrofti*. These worms were immediately transferred to warm saline solution. The worms were alive and their movements were somewhat sluggish. After about half an hour they did not show any movement and were apparently dead. They were then fixed in 4% formalin solution and stored away in 70% alcohol. Two of these worms were found to be males and the other two females. In general appearance they are similar to *Wuchereria bancrofti*. The female measures 55-56 mm. and the male 22-23 mm. in length. The tail of the male is spirally curved as in the case of the male *Wuchereria bancrofti*. Detailed morphological studies of the specimens are in progress.

44. Protozoal parasites enquiry under Major H. W. Mulligan, I.M.S. at the Pasteur Institute of Southern India, Coonoor.

The work of the enquiry during the year under review has been directed mainly to the study of the mechanism of immunity in protozoal diseases. Some preliminary experiments have also been carried out to determine whether the course of protozoal infections differs significantly in well-nourished as compared with ill-nourished hosts. A species of *Plasmodium* has been discovered in the blood of the Malabar squirrel. A brief outline of the work carried out is given below.

1. Immunity to protozoal diseases.

(*a*) *Passive immunity.*

Experimental work on monkeys infected with *P.knowlesi* and *P. cynomolgi* has shown that the course of these infections in previously uninfected *sinicus* monkeys can be modified by the passive introduction of material containing antibodies derived from infected monkeys at various stages of the infection. The materials so far employed for this purpose are blood plasma and juice extracted from the spleens of infected monkeys. Successful experiments have been carried out with plasma obtained from monkeys suffering from acute, sub-acute and chronic infections. Plasma obtained from superinfected monkeys has also been used with success. The results so far obtained suggest that protective antibodies are present at higher concentration in the spleen than in the circulating blood. It has been possible to make comparative studies of the efficacy of plasma and spleen juice at different stages of the infection in a small series of animals. Pathological material has been studied in an attempt to correlate the humoral and cellular agencies responsible for the production of immune responses. These studies have yielded interesting information particularly in regard to the cellular response to infection in splenectomised animals. The study of passive immunity in monkey malarial infections has so far been of a preliminary nature but the results already obtained have opened up a wide field for further investigation.

Experiments of a similar nature have also been carried out on white rats infected with *T.evansi*. The results so far obtained suggest that here also blood plasma from infected animals has some protective action as evidenced by a prolongation of the course of the infection in treated animals up to 8 or 10 days while in untreated animals it is invariably fatal after 3 to 5 days. Parasite counts made at regular intervals during the course of the infection show that a few hours before death there is a sudden drop in their numbers in the peripheral blood. Blood removed from the heart at this stage appears to give good protective action. The estimation of the percentage of dividing trypanosomes present in blood smears affords an index of the rate of reproduction of the parasites and, on this basis, the findings indicate that there is some protective action due to the presence of a reproduction-inhibiting antibody (*ablastin*). Spleen juice from infected rats appears to have little, if any, protective action. The regional concentration of trypanosomes which occurs in the lungs prior to death suggested that antibodies might be at higher concentration in these organs but protective experiments did not substantiate this hypothesis.

(*b*) *The influence of splenectomy on monkey malarial infections.*

Opportunity was afforded during the conduct of the work on passive immunity to study the effects of splenectomy on the course of malarial infections in monkeys. Splenectomy of *sinicus* monkeys suffering from latent infections with *P.cynomolgi* or *P.knowlesi* was found to provoke a relapse which was more severe than the primary attack. Intact *sinicus* monkeys usually recover spontaneously after infection with *P.knowlesi* but, if the spleen be removed prior to the introduction of the infection, the subsequent attack is invariably fatal and is frequently associated with haemoglobinuria.

(c) *The persistence of acquired immunity after the radical cure of malarial infections.*

A large series of experiments has been carried out to determine the persistence of immunity acquired to malarial infections in monkeys after the production of radical cure. These experiments have clearly demonstrated that immunity to homologous reinfection may persist for a considerable time after the original infection has been eradicated. In other words there is some "residual" as distinct from "concomitant" immunity in such infections. The degree and duration of this residual immunity is variable and appears to depend largely on the nature and amount of antigenic (parasitic) stimulation to which the host has previously been subjected.

2. THE INFLUENCE OF THE STATE OF NUTRITION OF THE HOST ON THE COURSE OF PROTOZOAL INFECTIONS.

Some preliminary experiments have been carried out in collaboration with the Nutrition Research Laboratories, Coonoor, to determine whether the course of protozoal infections differs significantly in well nourished as compared with ill-nourished animals. Failure to establish suitable protozoal infections in the white rat has retarded the study of this problem in an animal whose dietary requirements have been fully investigated. *T.lewisi* isolated from wild caught rats proved to be of such low pathogenicity for the stock white rats that it was difficult to follow the course of this infection. Passage of this parasite through splenectomised white rats did not enhance its virulence for intact rats. No evidence was obtained that the course of *T.lewisi* infections differed significantly in a series of well nourished as compared with a series of ill-nourished white rats. Further attempts along the same lines were made with white rats infected with *T.evansi* but in this case the parasite was so highly pathogenic for both groups that the results obtained could not be evaluated.

Some interesting results have been obtained, however, from the study of the course of *P.cynomolgi* infections in monkeys. The number of animals so far investigated is small as it was considered expedient to defer experiments of this kind until the monkeys which were to be used for the experiments had been on their appropriate diets for at least 6 months. These preliminary experiments have indicated that in monkeys fed on appropriate diets for a period of from 6 months to a year the course of *P.cynomolgi* infection is as severe in well-nourished monkeys as it is in ill-nourished monkeys.

3. SQUIRREL MALARIA.

A species of *Plasmodium* has been found to occur in the blood of the Malabar squirrel (*Ratufa indica*). All attempts to establish this infection in common laboratory animals such as the white rat, the rabbit, the guinea-pig and the monkey have been unsuccessful. It would obviously be a great advance if a malarial infection could be established in some small laboratory mammal and further attempts are being made to infect other animals more closely allied to the Malabar squirrel.

45. Collection of further data regarding cancer in India under Dr. Vishwa Nath, Department of Pathology, K. E. Medical College, Lahore.

In the survey of incidence of cancer in India and Burma which was completed last year, material and information which had collected in different institutions over many years was utilised. So far as autopsy records were concerned the data extending over a period of 40 years were taken into account. It was considered necessary to check the results obtained against the incidence of the disease at the present time. The same institutions from which information was collected for the all-India enquiry were approached for furnishing the data for the year 1939. Information for the half year ending June, 1939, has been received from most of the institutions. Figures will be complete when data up-to 31st December, have also arrived. It can be stated in general terms, however, that the data so far received are confirmatory of the findings published in the general survey both as regards the incidence of cancer of different organs of the body and as regards age distribution. A full report will be submitted after the facts concerning the year as a whole are in hand.

46. Typhus enquiry under the Director, Pasteur Institute, Kasauli.

The transmission experiments with trombiculid mites which had been planned for the typhus season of 1939 could not be carried out owing to failure of the breeding experiments.

It had been observed that Kasauli brown monkeys are sometimes infested with larval *Trombicula deliensis* but attempts to isolate typhus strains from these monkeys or from mites collected from them have so far been unsuccessful.

Various attempts to demonstrate typhus infection in pariah dogs have been unsuccessful.

Transmission experiments produced no evidence that bed-bugs are implicated in the epidemiology of XK typhus.

A number of experiments with wild rats and with trombiculid mites removed from wild rats have so far failed to yield evidence of XK typhus infection. Captain Buckland, R.A.M.C., has collaborated in this part of the work.

Laboratory observations on XK typhus strains isolated from human cases in the Simla Hills indicate that these strains are similar to the strains isolated in Malaya from cases of scrub typhus.

Samples of blood were secured from a number of febrile cases among local villagers at the season when XK typhus cases are believed to occur. Twenty out of 25 proved to be malaria and one a paratyphosus B infection. The other three were tested for typhus by animal inoculation and an XK strain of typhus was secured from one of these.

47. Filterable viruses enquiry under the Director, King Institute, Guindy.

VACCINIA VIRUS.

The investigations into the antigenic analysis of two strains of vaccinia virus, which was initiated last year, was completed during the period under report. The nature of the strains investigated and the technique adopted in the study were described in last year's report to the Scientific Advisory Board.

An analysis of the various results obtained shows a close identity in the antigenic composition of the two strains studied. The agglutination tests have revealed the identity of the heat labile fractions of the two strains. It was also found that the infectivity and complement binding antigens were intimately associated with the heat labile fractions of the elementary bodies. No appreciable differences were noted with cross complement fixation tests and virus neutralisation tests.

It would be interesting here to refer to the work of Downie, who investigated the relationship of the virus of cow-pox to vaccinia virus as is obtained in the routine production of calf lymph, and came to the conclusion that while there was a close immunological relationship between the virus of spontaneous cow-pox and the virus of vaccinia in current use in laboratories, a closer analysis revealed the fact that the antigens although similar were not identical. The cow-pox strains he used, however had been propagated only through 26 passages over a period of 10 months, and he admitted the possibility of a modification of the cow-pox virus towards a closer approximation towards the 'standard' vaccinia virus.

In the present work, the strain of vaccinia virus obtained from small-pox material has passed through 43 passages for a period of over 2 years, and as such its close approximation to the standard vaccinia virus is readily understood.

These results, however, establish the fact the strain now in use in the manufacture of vaccine lymph is identical in most respects with that adapted directly from variola virus. The practical applications of this finding are obvious.

VIRUS OF LYMPHOGRANULOMA-INGUINALE.

A study of the virus of lymphogranuloma-inguinale has been undertaken. The virus was successfully cultivated on egg membranes and carried through 5 serial passages. The immediate object of this investigation is to make Frei's antigen from egg membranes or from flask cultures. The antigen made from egg membranes was tried experimentally. Conclusive results were not obtained. The work will be continued.

VIRUS OF SAC-BROOD OF HONEY BEES.

At the request of the Agricultural Entomologist, Coimbatore, attempts were made to cultivate the virus of "sac-brood of honey bees". There is evidence to show that serial passages on egg membranes may be required before visible results are obtained on egg membranes. At present there is no experimental evidence to show that the virus has been successfully cultivated by this technique.

48. Epidemic dropsy enquiry, (laboratory section) under Major C. L. Pasricha, I.M.S., at the School of Tropical Medicine, Calcutta.

It was shown (Indian Med. Gaz. Vol. 64, 1939, p. 123) by feeding experiments in man that the oil expressed from the seeds of a weed *Argemone mexicana* leads to the development of signs and symptoms resembling those of epidemic dropsy. It is interesting to recall that Sarkar in 1926 implicated argemone oil in outbreaks of epidemic dropsy. Kamath in 1928 described an outbreak of epidemic dropsy in Ganjam district. Oil pressed from certain seeds (locally known as odismari seeds) was popularly believed to be responsible for the outbreak of the disease. Samples of odismari seeds were obtained and were identified by Mr. R. L. Badhwar to be the seeds of *Argemone mexicana* and the oil expressed from these seeds gave a positive nitric acid test for argemone oil. It is interesting to recall that Kamath in his report in 1928 in discussing the various theories wrote "Another idea gaining ground among the villagers is that the disease is due to some poison in adulterated gingelly oil. They allege that gingelly seeds are mixed with seeds of an agency plant called in Oriya "Odismari" and that the oil from latter seeds is poisonous. In support of this theory, they state that the pure oil of these seeds when applied to the body produces erythema and local swelling, and that, if internally administered, it is certain to produce the disease. Enquiries made from those who extract the oil bear out this statement". It is unfortunate that these two reports did not receive the attention they deserved.

During the present year the effect of the oral administration of argemone oil to laboratory animals was studied. Argemone oil is more toxic to laboratory animals than mustard oil, olive oil or liquid paraffin. When the argemone oil is heated till it "fumes" well (at 240° C for 15 minutes) the toxicity of the oil is not much more than that of bland oils such as olive oil or liquid paraffin. The heated oil gives a positive nitric acid test. Heating the oil at 100° C or 150° has no appreciable effect in its toxicity to animatls. If the results obtained in animals are confirmed by observations in man hen the heating of the oil before use would be a simple method of controlling the disease.

Argemone oil given by mouth to guinea-pigs or mice causes extensive degenerative changes in the liver and the kidneys. The lesions resembled those described following the administration of substances such as diethylene dioxide (dioxane). The changes in the liver in ahimals fed with argemone oil differ from those found in dioxane poisoning in that definite groups of hepatic cords drained by tributaries of the hepatic veins are not affected. The vascular changes in the kidneys occur both in the cortex and in the medulla in animals fed with argemone oil whereas with dioxane the kidneys showed hydropic degeneration of the cortical region only. The changes produced by argemone oil are more extensive than those observed with dioxane. It is hoped to extend this work and to include the changes produced by different fractions (saponifiable and non-saponifiable) of the oil. Certain preliminary experiments carried out with these fractions suggest that the fatty acids of argemone oil are more toxic than the whole oil.

The amounts of argemone oil required to produce symptoms in guinea-pig and man were calculated. In animals a loss of more than 10 per cent of the original weight with loss of activity, and roughening of the coat were taken as indicating toxic effects. Approximately 0.54 cc. of unheated

argemone oil per 100 grams of body-weight is required to produce toxic effects in guinea-pigs and 0.95 cc. of the oil heated at 170° C and 1.23 cc. of the oil heated at 240° C are required to produce toxic effects. In man approximately 0.88 cc. of argemone oil per 100 gram body-weight produced ill effects.

Outbreaks of epidemic dropsy in two closed communities were studied. In one outbreak 37 or 15.2 per cent of the 242 body inmates developed epidemic dropsy. Of the 37 boys affected 17 or 46 per cent had sarcoids. The mustard oil used was drawn from one source. The seeds from which this oil was expressed were from a consignment which had been received a year previously. The ration of oil was two ounces of the oil per day per boy. This oil gave a positive nitric acid test for argemone oil. The intensity of colour suggested that argemone oil was present in about 1 per cent concentration. (Indian Medical Gazette, December 1939).

In the other outbreak 15 or 56 per cent of 27 Indian nurses (whose food was from one kitchen) developed epidemic dropsy. The food-stuff supplied to the nurses' kitchen and the hospital to which these nurses were attached was from the same source except for the mustard oil. The oil used in the nurses' kitchen contained approximately 3 per cent argemone oil whereas the oil used in the patients' kitchen was free from argemone oil. The rice used in the nurses' kitchen and for the patients was from the same source and was of parboiled variety without any opacities.

In both these outbreaks the use of mustard oil was stopped after which no fresh cases occurred.

The sedimentation rate of red blood cells, the cell volume and the haemoglobin content were estimated in a series of 74 cases of the natural disease and in 8 persons in whom symptoms resembling those of epidemic dropsy were produced, following the intake of mustard oil containing argemone oil.

The sedimentation rate of red blood cells is increased in epidemic dropsy. There was not a single case of established epidemic dropsy in which the sedimentation rate was not increased. As a rule the lower the rate, the severer the disease and the worse the prognosis.

Fibrin-nitrogen content was estimated in a series of 20 cases of epidemic dropsy. The sedimentation rate, the cell volume and the fibrin content are interrelated. An increase of fibrin leads to an increase in the sedimentation rate provided the cell volume is constant. With an increase of cell volume the sedimentation rate is decreased provided the fibrin content is constant.

It is not the mustard oil per se or any disease of the mustard plant or seeds that leads to the development of noxious substances in the oil but there is an adulteration, (probably an accidental adulteration but one which is preventable) that is responsible for the widespread outbreaks of epidemic dropsy. The evidence for the toxic factor is strong and is admitted by experimental workers, but what exactly the factor is or how it acts is not as yet known. Is its action associated with the absence or scarcity of vitamins or other essential factors (possibly mineral) in the diet which allow the development of endogenous intoxication, thus bringing this disease in the beri-beri or food deficiency group of diseases? There are not very definite points of distinction between the two and a number

of arguments can be advanced to support this hypothesis. The plant *Argemone mexicana* grows widely. The disease occurs sporadically and occasionally assumes epidemic proportions. The reasons for these outbreaks still remain to be determined, whether they are associated with any biological factors associated with the plant itself or whether the outbreaks are associated with dietetic factors peculiar to or operating in association with the epidemic phase of the disease. Does rice play any associated role? It is known that rice leads to the exacerbation of oedema in an individual suffering from the disease. These are some of the questions that remain to be solved. From the purely laboratory point of view the most interesting line of work in progress is the effect of the oil and its different fractions on animals and whether lesions or death can be prevented in any way.

49. Epidemic dropsy enquiry (field section) under Dr. R. B. Lal at the All-India Institute of Hygiene and Public Health, Calcutta.

Field investigation.

1. *Estimation of the value of differential tests.*

In last year's report 2 physical and 2 chemical tests to differentiate between samples of mustard oil associated with epidemic dropsy and those not so associated have been described. Further work was carried out to assess the value of those tests. The final results are shown in table I.

Altogether 320 samples of oil with epidemiological history were collected by the field staff and health officials. Of these 244 samples were accompanied by satisfactory epidemic history.

Table I.

Test.		Epidemiological history and toxicity positive.	Epidemiological history and toxicity negative.	Epidemiological history and toxicity indefinite	
				Suggestive.	Not suggestive.
Nitric Acid	Positive	21	2	38	58
	Negative	2	21	16	86
Cupric Acetate	Positive	21	0	37	50
	Negative	2	23	17	94
Fluorescence	Positive	22	11	49	98
	Negative	1	12	5	46

2. *The incidence of Argemone mexicana seeds in stocks of mustard seeds.*

Mention was made last year of an investigation to determine the extent to which *Argemone mexicana* seeds are found in samples of mustard seeds stocked for purposes of pressing oil. Further work on these lines was carried out. The results are summarised in Table II.

Table II.

Percentage of argemone seeds ..	Nil	—1	—2	—5	—10	above 10
Number of samples ..	179	150	24	27	6	10

Further analysis showed that it was only in certain varieties of mustard seeds that the argemone seeds were found in appreciable quantities. These varieties are known as Torai and Rai (Brassica juncea). The reason is obvious *e.g.* that these two varieties of mustard seeds are light black in colour resembling that of argemone seeds and the size of the seeds is also very much the same.

3. *Experiment to note the effects on consumption of mustard oil pressed from mustard seeds containing an appreciable quantity of seeds of Argemone mexicana.*

Daily examination of mustard seeds ready for crushing in an oil mill was made to estimate the percentage of *Argemone mexicana* seeds. A stock containing 9% *Argemone mexicana* seeds was discovered after a long wait. This supply of the seeds was crushed in a specially cleaned crushing mill and the oil was stored in a cleaned tank, after dilution for trade purposes, with oil from other consignments of mustard seeds. The ultimate mixture contained 1.75% *Argemone mexicana* oil. Similarly a supply of oil obtained from stock of mustard seeds free from *Argemone mexicana* seeds was stored in another cleaned tank. The two supplies were filled in tins having distinguishing marks and were consigned to distant isolated communities in which there had been no outbreak of the disease for some time. A close watch was kept on the people consuming the oil for over a month but no cases were detected, except in one well-to-do family which had consumed a full tin of the contaminated oil. The absence of cases amongst other families may be accounted for by the fact that the oil contained less than 2% of argemone oil as compared with 5% in Rangpur oil which had given clear cut results in a human experiment and that the people being poor could only afford to use the oil in very small quantities and that too irregularly.

4. *Relation between the concentration of argemone oil in the mustard oil and the development of clinical symptoms.*

From the above experiment as well as from our previous experience it is evident that symptoms of the disease do not develop unless a sufficient amount of the toxic substance is consumed. As may be seen from table II small quantities of *Argemone mexicana* seeds are found in a large percentage of stocks of mustard seeds in the oil mills. The question therefore arises whether it will be justifiable to discard these supplies since the habits of the people vary considerably with regard to the amount and regularity with which they use mustard oil specially in regard to the constancy of the source, it is not possible to lay down a limit beyond which contamination with argemone oil may be considered dangerous to health. However for the reasons stated above the quantitative aspect of the problem assumes considerable importance and some legal standards may have to be introduced. For this purpose a quantitative test of the amount of reacting substance in the oil was developed. This consisted of a colorimetric estimation of the nitric acid reaction as tested by Pulfrich's photometer under standard conditions. An investigation was made in certain hostels in

Calcutta to see the relationship between the development of clinical symptoms and the quantity of oil of known colorimetric value and duration of consumption. Although the investigation has been carried out throughout the year neither symptoms of the disease have been observed in this group nor has oil containing appreciable quantities of the reacting substance been discovered.

5. *Chemical investigations* :—

(*i*) Development of the quantitative test :—Reference has already been made to this test. The standard conditions worked out for the determination of extinction co-efficient were as follows :—

(1) Wavelength—4600 A U.
(2) Period for completion of the test 20 minutes.
(3) Thickness of the cell 0.5 to 1 centimetre according to the concentration of the reacting substance.
(4) Dilution ranging from 1 in 1 to 1 in 4 according to concentration. A gauging curve expressing the coefficient of extinction with the quantity of reacting substance and roughly with the concentration of *Argemone mexicana* oil has been worked out. Thus the amount of contamination in the oil can be read off directly.

(*ii*) Effect of light and air on the concentration of the reacting substance :—

It was found that the reactivity of mustard oil associated with epidemic dropsy as also of mixtures of *Argemone mexicana* oil in pure mustard oil in various dilutions was reduced on exposure to light particularly in the presence of air. Temperature was not a factor. The experimental results are shown in table III.

Table III.

Nature of sample	Control reading	Exposed to direct sunlight for 5 hrs in contact with air	Exposed to direct sunlight for 5 hrs in the absence of air	Exposed to diffused light for 3 months in contact with air	Exposed to diffused light for 3 months in the absence of air	Kept in the dark for 3 months in contact with air	Kept in the dark for 3 months in the absence of air.
Pure jail produced mustard oil	0	0	0	0	0	0	0
Mixture of 5% argemone oil in pure mustard oil	4.9	1.68	3.4	2.4	2.3	4.7	4.6
Pure argemone oil (diluted after treatment to form a 5% mixture with pure mustard oil).	4.9	2.9	5.0	2.8	4.8	4.76	4.7

It was shown that the reduction could not be attributed to allylisothiocyanate content of the oil.

(*iii*) In last year's report reference was made to an organic crystalline compound from *Argemone mexicana* oil and mustard oil associated with the disease. On studying the melting point and microchemistry it was found that the results were not constant. Using another technique it is now possible to obtain pure white rhomboidal crystals giving a constant melting point of 189° C and density of 1.4 at 30° C. This substance satisfied all the differential tests. The crystals leave no ash on incineration. They are sparingly soluble in absolute alcohol but insoluble in strong or dilute alkalies. They dissolve in dilute hydrochloric acid and in most organic solvents. Microchemical analysis suggests an empirical formula, $C_{19}H_{15}O_4N$. Further work on the nature of this substance is in progress. It is probably alkaloidal in nature as shown by Mayer's and other tests for alkaloids. The properties of this substance as given above do not correspond to those of berberine or portopine the two alkaloids which have been described in *Argemone mexicana* plant. It has been found possible almost to free the *Argemone mexicana* oil of the reacting substance by extraction with dilute hydrochloric acid.

6. *Biological test.*

Further progress in the elucidation of the toxic substance is greatly handicapped because of the lack of a suitable biological test. Clinical test on human subject is not easily available. Reference was made to the skin test and test on laboratory animals in last year's report. Further feeding experiments on cats were carried out. In these tests the problem as to the correspondence or otherwise of contents of oil in reacting and toxic substances was also studied.

Cats were selected for the feeding experiment as the previous work had shown that these animals were somewhat susceptible to argemone oil.

The animals were divided into 5 groups as follows :—

(*a*) Fed on 5% argemone oil in pure mustard oil.

(*b*) Fed on 5% argemone oil in mustard oil, the argemone oil being previously altered by exposure to sunlight for a long time so as to reduce reactivity to about one-tenth of the original.

(*c*) Fed on mustard oil containing 5% argemone oil from which HCl soluble substance had been removed, final mixture containing only about one-fiftieth of the reacting substance of argemone oil.

(*d*) Fed on pure mustard oil containing the white crystalline substance obtained from argemone oil, the mixture giving Pulfrich reading equivalent to 5% argemone oil.

(*e*) Control group—fed on pure mustard oil.

Animals in group (*a*) suffered definite damage to their health. They suffered from anaemia, emaciation and loss in weight. The animals in the other groups gained considerable weight and showed no evidence of deterioration in health. Skin snips were taken from the animals at fortnightly intervals. Group I cats showed proliferation of capillaries in the skin. The capillaries, however, did not consistently show any dilatation as is commonly found in affected human skin. Cats in other groups did not show such changes but there was dilatation of capillaries in certain organs. If proliferation of skin capillaries is taken as a criterion of specific toxic

effect, the above results would indicate that the organic crystals are not toxic and that both the reacting and the toxic substances are removed by dilute HCl and destroyed by the action of light on the oil.

50. Kala-azar enquiry under Dr. R. O. A. Smith at the Darbhanga Medical School, Bihar.

The personnel and equipment of the enquiry was transferred from Calcutta to its present venue in May 1939, and the work done may conveniently be reported under the following heads :—

(1) A study of the epidemiology of kala-azar in Bihar.

(2) Transmission experiments with *P.argentipes* as well as by methods not involving the agency of insects.

Epidemiological study.

Since experiments to incriminate the sandfly *P.argentipes* as the vector of kala-azar had not yielded conclusive results up to date, it was proposed that further information regarding the natural spread of the disease should be collected and to this end a schedule was prepared for recording in a convenient form all particulars of kala-azar patients. For various reasons it was not possible to depend solely on the patients attending the out-patient department of the hospital for this purpose and an area was selected a few miles away where the disease was fairly prevalent and where a treatment centre was very kindly opened by the District Health Officer. This centre has attracted patients from over 40 villages within a radius of about ten miles, and so far over 300 cases have been diagnosed and treated. The schedules of over 200 patients have already been completed, and as soon as a sufficient number is available they will be sent to an epidemiologist for examination.

One village in this area has been selected for special attention. According to a census made by us there are 409 persons in the village living in 92 houses ; 24 cases of kala-azar have been detected among these people this year ; particulars of these cases, their homes and family members have been recorded with the object of determining the incidence of kala-azar next year among the persons in closest contact with the cases of the disease now existing in the village. This village is being visited regularly by the assistant in-charge of the centre and all cases of fever due to any cause are carefully investigated and noted against periodic visits from a member of the staff of the enquiry.

From general observation it may be mentioned that no correlation of the distribution and intensity of kala-azar and damage caused by the Bihar earthquake of 1934 has yet been found. It is however interesting to note that the area under investigation, where the disease may be said to be now fairly prevalent, was the scene of a virulent and widespread

epidemic of malaria during 1936 and 1937. Blood smears from 127 persons in this area, other than those giving a positive aldehyde test were examined. Malaria parasites were found in 12 and L. D. bodies in one case. In another area visited recently, of 36 blood smears examined, malaria parasites were found in 30 and M. T. in 21 of them. The sera of 10 other cases were examined for kala-azar, and all ten gave negative aldehyde test. The subsequent history of these villages now in the grip of an epidemic of malaria may yield interesting and important results.

Other than sandflies and mosquitoes none of the larger blood-sucking diptera were found. Lizards and spiders of many species were very numerous and the impression formed was that these natural enemies of insects are more prevalent in these villages, than in those of Bengal or Assam. Five hundred and nine sandflies, *P.argentipes*, collected mostly from village homes in the vicinity of the treatment centre have been dissected and examined for the presence of natural infections with *L.donovani*. It was in the month of September only that infected flies were detected. Of 154 collected that month three were found with flagellates.

Post-kala-azar dermal leishmaniasis does not seem to be very common; only 10 cases, mostly of the depigmented form, have so far been seen.

During this period 954 aldehyde tests have been carried out on patients attending the out-patient department of the hospital. Of these 409 have given positive results.

Transmission experiments.

With a view to selecting patients discharging *Leishmania* in their nasal and pharyngeal secretions for experiments in transmission not involving an insect vector, smears from 151 patients were examined. In two cases only were parasites found, but in such scanty numbers that they were not considered suitable for the purpose. This work is being continued.

P.argentipes were bred out in the laboratory in June and a change in the method of feeding and keeping the flies was made in the hope of obtaining better transmission results. Instead of giving them successive blood meals as was the routine in previous years, the sandflies were maintained on raisins till the tenth day after a preliminary blood meal on a kala-azar patient, and then offered a second blood meal on an experimental animal. The first infective bite was delivered by the flies on the tenth day instead of the sixth or seventh as was practised in previous experiments. By this method of handling the flies not only did about fifty per cent of them survive for delivering infective bites but a considerable saving of labour was effected.

In a certain number of infected flies kept by this method a condition of 'blocking' has been observed. These flies were seen to pierce the skin of the animal and to endeavour for appreciable lengths of time to suck up blood without being able to obtain any but the merest trace on occasions. The dissection and examination of such flies showed that the proventricular fold was almost completely obliterated and the oesophagus greatly distended with flagellates. Two papers, one on the new method of maintaining sandflies and the other on the phenomenon of 'blocking' have been prepared and accepted for publication.

Twenty-five animals have been placed under experiment this year. Of these 10 mice have been artifically inoculated with flagellates from sandflies fed on raisins, and 10 mice and five hamsters have been exposed to the bites of infected flies. Of the latter five mice and two hamsters have died. L. D. bodies were present in the smears of the liver and spleen of both hamsters. The incubation periods in respect of these two hamsters were six and ten weeks respectively. In the former, parasites were not numerous but the smears of the liver and spleen of the second showed a very heavy infection. In the four successful transmissions obtained by the bites of infected sandflies previous to 1934 the incubation periods in the hamsters varied from a year to eighteen months and two at least of them were diagnosed as the result of cultures. No L. D. bodies were found in the smears of the liver and spleen of the mice, but as these organs were not cultured owing to sepsis following decomposition, the results in respect of the mice are incomplete.

The presence of L. D. bodies in the smears of the liver and spleen of the hamsters after such short incubation periods is further strong evidence of the sandfly transmission of kala-azar.

51. Toxicological and medicolegal enquiry under Rai Bahadur Dr. K. N. Bagchi at the Medical College, Calcutta.

This enquiry has been undertaken to find out the normal limit of poisonous metals such as lead, copper and arsenic in human excreta, tissues and foodstuffs for purposes of comparison and expression of opinion in fatal cases of poisoning by these metals.

(a) Lead in normal urine and faeces.

Large numbers of samples of urine and faeces of healthy persons of all classes of people, not exposed to abnormal lead absorption, and also of those engaged in industries connected with lead, were examined. The normal range was found to be much below that of Europeans and Americans. The result has been of immense help in diagnosing mild cases of chronic lead intoxication among the Indian lead workers, especially those who claim compensation under the Workmen's Compensation Act. It has further been demonstrated that the findings of the research workers on this line in the West are not applicable to Indians.

(b) Lead in normal tissues.

All normal tissues including brain but excluding ovary, contain variable amounts of lead. The absence of lead in healthy ovaries indicates the susceptibility of germ cells to the poisonous action of lead which is known to produce repeated miscarriages, still-births or sterility in women engaged in lead industries. The testis, uterus and placenta, on the other hand, are found to contain 0.4,0.47 and 0.36 mg respectively per kilo of fresh materials. The foetal tissues do not show any affinity for lead although it is believed

otherwise. In fatal cases of acute red lead poisoning, the maximum amount of lead found so far in liver is about 17 mg while the maximum in a healthy liver is 0.82 mg.

It is interesting to note that of all tissues, hair is most remarkable in possessing large quantities of lead—much larger than what is found in bones or in teeth which are regarded as lead depots in the human system. About 200 samples of hair, from all calsses of people, were analysed and in one sample, as much as 508 mg of lead was found. The amount of lead appears to vary with the colour of hair, or in other words, lead may have something to do with the production of pigments which characterise hair of different nationalities and races. The following tables may give an idea about the result of this investigation :—

Table I.

	Men	Women
Europeans (including Jews and Anglo-Indians) ..	20.8	18.4
Indians	28.0	114.5
• Bengalee Hindu	26.7	180.9
Bengalee Muslim	42.4	50.4
Punjabi	20.2	45.5
Madrasi	22.7	..
U. P. & Behar people	21.6	..
Others (Parsee, Oriya, Marwari, etc.) ..	20.0	26.3

Table II.

	Minimum	Maximum
Deep black hair (Bengalee Hindu women) ..	170.0	508.0
Brown, auburn and other shades (Europeans resident in Calcutta) ..	9.0	16.5
Grey—containing 0 to 25 per cent of brown or black hair (Europeans & Indians)	3.0	21.0

The source of such abundance of lead in the hair of Bengalee Hindu women could be traced to vermilion which they use on the hair parting. Pure vermilion is red sulphide of mercury imported from China but the cheap bazar vermilion is either red lead mixed with a red synthetic dye or red sulphide of mercury adulterated with red lead. The urine and faeces of some of these women gave very high lead figures (about 10 times the normal figure) indicating the absorption of lead through the scalp.

(c) Lead in food and water.

About 150 samples of common foods-tuffs, of both animal and vegetable origin, and a few samples of potable water from tube well, tank, shallow well and the water supply of Calcutta, were examined. The lead content of some of the important articles of diet was as follows :—mutton 1.32 ; beef 0.15-0.3 ; goat 0.45-2.4 ; egg 0.26 ; Rui fish 0.84-1.2 ; lobster 0.24 ; wheat flour 0.28 ; dals (five different kinds) 0.04-0.28 ; potato nil-0.16 ; cabbage 0.24 ; cauliflower 0.2 ; tomato 0.28 ; pea 0.12 ; mango

0.07; orange 0.44; guava 0.24; butter 0.48; ghee 0.56; mustard oil 0.45-2.08; coconut oil 1.26; brandy 0.06; rum 0.024; beer 0.026 mg per kilo or litre.

The following foodstuffs did not contain any lead :—rice (two different kinds), onion, grapes, coconut, kai fish, hilsa fish, sea fish (sole and pomfret), lichi, radish, papaya, red pepper, soya beans, cheese, banana, etc.

The samples of drinking water gave 0.001 to 0.002 mg of lead per litre.

These findings will be of some help in establishing the relation between the concentration of lead in tissues and the total intake of lead in food and drink and its excretion through the usual channels of elimination.

(d) Copper in normal human tissues.

Apart from its biochemical importance, the investigation has been found useful in medicolegal work. In fatal cases of acute copper sulphate poisoning which is fairly common in Bengal, the quantity of copper estimated in necropsy materials, has so far not exceed 37 mg per kilo. The information about the copper content of normal tissues helps the medical jurists to express definite opinions in such cases of fatal poisoning. The maximum amount of copper found in healthy tissues, liver for example, of an adult person is 12 mgm per kilo.

The foetal tissues seem to possess a special affinity for copper, for instance, the liver of a seven month foetus gave as much as 37 mg per kilo. Copper thus differs in this respect from lead and arsenic which are not retained in excess in foetal tissues. On the other hand the copper content of adult tissues in cases of acute copper sulphate poisoning is much less than in acute arsenic poisoning.

(e) Arsenic in food.

The investigation into the arsenic content of human tissues and excreta was completed and published in 1937. The present enquiry has therefore been directed to the determination of arsenic in common Indian foods-tuffs. It is likely to be completed by the end of the current year. The following figures (mgs of As as $As_2°3$ per kilo of fresh material) would give an idea about the arsenic content of some of the common vegetable foods-tuffs :—rice 0.2; gram 0.12; onion 0.016; garlic 0.13; spinach 0.029; cauliflower 0.036; cabbage 0.128; red pepper 0.02; potato nil; radish 0.008; banana 0.04; brinjal nil; guava nil; soya bean nil; papaya 0.032; coconut 0.04; tea 0.18 to 0.21.

(f) Hydrocyanic acid in linseed flowers.

Fatal cases of hydrocyanic acid poisoning in cattle after grazing in fields where linseed crop begins to flower, are fairly common in Bengal and Bihar and possibly in other linseed growing provinces. The maximum amount of hydrocyanic acid (0.692) is found in linseed flowers just after fertilisation.

52. Enquiry on bone-marrow and studies of some of the secondary anaemias of India under Dr. H. N. Chatterjee at the Carmichael Medical College, Calcutta.

Enquiry on bone-marrow.

Further specimens of bone-marrow numbering 42 have been secured making a total of 147. The cases include almost all the important diseases of India. Amongst these may be mentioned malaria, kala-azar, cholera, epidemic dropsy, diphtheria, tuberculosis, enteric fever and malignant diseases. The specimens have been sectioned and stained with the different methods of examination.

A method of counting the total number of cells in sections of the bone-marrow has been worked out and is being applied to the study of the above specimens. This gives quantitative information about the activity of the bone-marrow.

Following the technique described by Dr. L. E. Napier, sternal punctures have been performed in 15 postmortem cases, in many of them within an hour of death.

The information obtained from the study of this marrow from a spongy bone and consisting of a very complex structure is being compared with the compact tissue of the femoral bone-marrow.

The previous study that was being carried on about the bone-marrow of cholera has been considerably enlarged.

Study on secondary anaemias.

Work has been carried on in various directions. Complete haematological examinations have been performed in 58 more cases of different varieties of secondary anaemias including those due to kala-azar, malaria, haemorrhage, anaemia of pregnancy, cancer etc.

Therapeutic investigations have been carried out in 14 cases of anaemia of pregnancy with the sterols along with two control cases. Although the number is small and the therapeutic weapon new, the results are rather promising and justify further work.

Previous biochemical investigations on this type of anaemia have been further carried on, along with normal pregnancy cases. The examinations include blood cholesterol, ester cholesterol and free cholesterol of the blood, Van Den Bergh, serum bilirubin, icterus index, albumin, globulin, euglobulin and pseudoglobulin and estimation of gastric acidity.

Similar biochemical investigations have also been carried out in 32 cases of secondary anaemias.

The previous study of the leucocyte counts and their relation to the diagnosis, prognosis and the erythrocytic picture has been further extended.

It has also been possible to make estimations of the lecithin fractions of the blood (White-horn's method) in Indian women under normal conditions (25 cases) and in pregnancy (25 cases).

53. Enquiry on thrombo-angeitis obliterans under Dr. R. N. Cooper at the Seth G. S. Medical College, Bombay.

During the period under review, 37 fresh cases were admitted for investigation and 6 old cases returned for treatment. Of the 37 cases, males numbered 35 and females 2. Twenty-seven were Hindus, 7 Mahommedans, 1 Anglo-Indian and 2 Hindu females. On detailed examination of the total number of cases, 3 were found to be of syphilis, 2 of arteriosclerosis and 5 were of uncertain diagnosis.

The enquiry aims at a detailed study of the factors involved in the occurrence of thrombo-angeitis obliterans, its clinical manifestations and its reaction to various lines of treatment. A thorough clinical examination with the help of various instruments, such as the pyrometer and the oscillometer was carried out.

Laboratory investigations included, besides those done during the previous year, examination for chlorides and cholesterol, the sedimentation rate and blood culture. Each time attempts were made to eliminate all cases other than the condition under investigation. For the present we have planned out and begun animal experiments. Biopsy material is being injected intramuscularly, intravenously, periarterially and intracerebrally into the experimental animal selected, the rhesus monkey. Sublethal doses of nicotine are also being injected subcutaneously over long periods. This will enable us to evaluate the aetiological factors concerned, especially infection by micro-organisms and viruses, and the part played by the use of tobacco.

The pyrometer was used in each case for observation of the variations in surface temperatures under diseased and experimental conditions. The readings have not been satisfactory and do not compare well with those recorded by American workers.

Graphical records have been obtained by the tensiograph under different experimental conditions. Interesting information was obtained by taking the records in positions of limbs when in comfort and when in extreme pain. Also before and after surgical procedures and venous ligation. More interesting records have been obtained when patients and normal persons were smoking different brands of tobacco.

Arteriography by the injection of a new product "Perabrodil" in a restricted field of circulation was done in eight cases. Besides its diagnostic value, the producers claim that it has therapeutic effects. Its only advantage so far evisaged is that it does not precipitate gangrene in the limb as found with the use of uroselectan B.

Pathological investigations have been undertaken along various lines. It has not been possible to obtain any organisms from a freshly dissected lesion of migrating phlebitis. Detailed histological examination of the blood vessels and nerves has revealed that an inflammatory process is in progress. From post-mortems done in three cases, the disease does not appear to be limited to the vessels of the extremities only. The visceral vessels are also involved though to a less extent and the condition is usually associated with arteriosclerotic changes.

Hormone preparations were administered by injections with no good results. Buergers exercises have been found to be beneficial in very

early cases only. Passive hyperaemia was tried, the pressure being applied to such a height as to reduce the rest pain to a minimum. Venous ligation was tried in 4 cases, but yielded fair results in one case only.

Arterial sympathectomy and lumber ganglionectomy did not give satisfactory results and multiple amputations were done at later dates. The value of hypertonic saline is definitely proved and some cases do very well with repeated injections of 300 c.c. of 5% saline solution. There is arrest of gangrene and an early establishment of the line of demarcation.

Vitamin feeding and high cholesteral diet have been tried with no good results. Blood cholesterol and sedimentation rate were estimated in these cases with no change. In all cases before treatment and feeding, the readings were found to be slightly low and the sedimentation rate rapid.

Prontosil and uleron were tried out but no good effects were obtained.

The whole picture of thrombo-angeitis obliterans suggests that some infection is at work. The animal experiments started by us promise some interesting results. One of the animals died 7 days after the injection of the defibrinated blood of a case of thrombo-angeitis obliterans, the control animal being quite healthy.

With more time at our disposal to continue these experiments we are hopeful that encouraging results would result.

So far the problem has been complicated and with future work, it is possible to get useful information which may fit into the final picture of this maiming disease.

54. Algal enquiry under the Director, King Institute, Guindy.

The work during the year 1939 related to :—

1. the determination of the forms and species of algae occurring in different types of water under varying seasonal and climatic conditions ;
2. the study of the relationship of the chemical constituents of the water and of the environments to the algal flora that predominate at different seasons of the year ;
3. the study of the effect of different species of algae on prefiltration and filtration processes ;
4. the study of the relative merits of various algicides when used separately and in different combinations and also of the effective doses of these algicides consistent with economy ; and
5. the detailed chemical and other analyses referred to in the last report (1938) to the Scientific Advisory Board.

The following water supply sources were thus studied in detail :—

Red Hills Lake, Madras.

The failure of the north-east monsoon (1938) and the very scanty rainfall during 1939 in the catchment areas of the Red Hills Lake and its feeder sources resulted in a considerable lowering of the level of the lake, such as has not occurred for several years past. The conditions obtaining in the lake, particularly from the point of view of an algal enquiry have, therefore, been abnormal, in spite of which valuable data have been collected. Thus, the close relationship noticed between the growth of a diatom (*Synedra*) and the silica content of the water, the total absence of nitrates and nitrites both in the lake water and in the bottom sediment of the lake, and the fact that no thermal stratification could be demonstrated in the lake in spite of the greatly lowered level, consitute some of the findings which are of far-reaching, importance, particularly because they differ from the findings of other observers in the temperate regions.

The lake received, however, an influx of fresh water almost to full tank level by the end of November 1939. The systematic study of the algal flora under normal conditions has, therefore, been prompty started again.

Ootacamund.

Green algae predominated in summer, gave place to desmids after the rains but re-established themselves by March, in the Marlimund reservoir at Ootacamund. In the Dodabetta reservoirs, a preponderance of diatoms followed a period of increased silica content, while the calcium and iron contents of the water were found to influence the growth of the green algae (*Peridinium*).

Rajahmundry.

At Rajahmundry difficulties in alum coagulation (prior to filtration) were experienced with the Godavari river water when *Melosira* predominated. A similar phenomenon was observed (in the laboratory experiments on alum coagulation) with the Red Hills Lake water of the Madras City where a different species (*Phormidium*) was responsible for the interference.

Adoni.

A field investigation was carried out during the year at Adoni where as a result of profuse growths of *Clathrocystis*, the municipal slow sand filters clogged in 3 to 4 days and chlorination became exceedingly difficult. Different algicides both alone and in different combinations and strengths were tried and the best one suitable for use in this particular water was determined.

Guindy.

Experiments were also initiated in October 1939 for carrying out daily observations over a whole year on the algal flora and the chemical and physical features of a river water stored in a large open artificial masonry reservoir within the King Institute compound. These controlled experiments are intended to throw light on various problems connected with the ecology and periodicity of algal forms occurring in stored waters.

MADRAS CITY.

Algae-laden waters from tanks in and around the city of Madras are also being studied with reference to the use of the best algicide or a combination of algicides suitable for the different species of algae met with in different seasons of the year. This will greatly assist in determining the line of treatment required for drinking water tanks in rural areas.

GENERAL.

The findings recorded during the year would appear to be at variance with those recorded by the British Fresh Water Biological Association at Wray Castle and in the Metropolitan Water Board's laboratories at London. The British workers have laid considerable stress on the '*Nitrogen balance*' in large bodies of water and in the sediment of lakes, as being almost the exclusive index of biological productivity. In none of the waters that have been studied in the Madras algal enquiry have nitrates or nitrites been found. Phosphates are also entirely absent. The factors governing the growth of algae and influencing their life cycles in the tropics would, therefore, appear necessarily to be very different from those in the temperate zones. No systematic investigation seems to have been made so far into *this aspect* of the problem anywhere in the tropics. The role of silica, iron, calcium and organic matter in promoting algal production in the tropics is, therefore, being studied.

A study of the various methods in vogue for quantitative estimation of plankton collections has been made and as none of them has given entirely satisfactory or consistent results, attempts are being made to evolve a new method or a modification of an old one, which would be suitable for making an accurate numerical estimate of the forms present. It would then be possible to determine the time when particular forms of algae are likely to multiply enormously and assume troublesome proportions.

55. Sandfly fever enquiry under the Director, King Institute, Guindy, Madras.

The field work of the enquiry was carried out in Peshawar and the laboratory work both in Guindy and in Peshawar.

FIELD WORK.

Commencing early in March, a search was carried out at Peshawar daily for sandflies. Some flies of the *P.minutus* group were caught on March 9, and the first *P.papatasii* on March 22. Later in the year, *P.papatasii* could not be found out of doors after November 12, but a few isolated specimens were caught in various indoor rooms as late as November 24. A consideration of meteorological tables for Peshawar at these times of appearance and disappearance showed that the daily range of temperature

was about the same *i.e.* maximum between 75° F and 80° F and minimum rather less than 45° F. These would seem to represent 'threshold' temperatures, below which adult sandflies cannot survive, or at least, do not emerge from the pupal stage. The relative humidity about both these dates was between 40% and 45% which is the usual figure for Peshawar throughout the year except on very occasional wet days.

Breeding of flies in the laboratory was carried out and as the weather became warmer, a gradual shortening of the time required for complete metamorphosis was noted. In July and August detailed observations were made of the number of days passed in each stage, and in eight batches of flies the periods were as follows :—egg 4-6 days, larva 14-17 days, pupa 5-11 days ; total 26-30 days. A batch put down on September 24 required a total of 42 days made up as follows :—egg 6 days, larva 17 days, pupa 19 days. It would appear that the chief variation is in the pupal stage.

At the suggestion of Major-General W. H. Hamilton, I.M.S., D.D.M.S., Northern Command, the application of a coat of tar to the walls of buildings for one to two feet in each direction at the junction of wall and ground, was tested as a means of reducing the sandfly population of a building. Subsequent surveys over a period of ten weeks indicated that if the tar is applied to the exterior alone, there is no appreciable reduction, but if also applied to the interior, the number of sandflies present is reduced by 25% to 50%. A coat of crude oil applied to the exterior alone produced no appreciable reduction.

A large number of experiments was carried out to investigate the effect on sandflies of various insecticidal solutions. Of "kerosene base" solutions satisfactory results were obtained with "Lethane" and three pyrethrum extracts *viz.*, "Stafford Allen's Liquid Extract (Pyefly)", "Pyrocide 20" and "Pyractone". Of these, solutions, "Lethane" is the most economical, and investigations into the exact dosage required gave constant results, *e.g.* it was found that if a closed room be sprayed, either with an atomiser or with a simple type of hand-spray, an atmospheric concentration equivalent to 7.0 c.cs. of pure solution per 1,000 cubic feet of air space will kill all sandflies present, and a concentration as low as 0.8 to 1.0 c.c. per 1,000 cubic feet was found to produce a reduction of from 50% to 60% in the number of sandflies present twelve hours later. The pyrethrum extracts are more costly and dosage appeared to vary, sometimes very considerably, with different dilutions. None of the solutions appeared to exert a repellent effect which was still perceptible at the end of twenty-four hours, and most satisfactory results were obtained by spraying rooms at dusk. For this purpose, weaker solutions than those in routine use were found satisfactory *e.g.* 1/66 "Lethane" (as compared with the usual 1/33) and 1/100 "Pyefly" (as against the usual 1/75). Two "water base" insecticides, "Deskito" and "Multicide" were also tested. "Deskito" in 7% solution is lethal to sandflies on direct contact, but was found to require an atmospheric concentration equivalent to 20 c.cs. of pure solution per 1,000 cubic feet to kill them indirectly. "Multicide" was found to have little effect on sandflies under the conditions of test. An atomiser of the "Phantomyst" type was used in some of these experiments, but does not appear to have any real advantage over hand sprays, provided the latter are properly used.

An effort is being made to determine the maximum altitude at which *P.papatasii* is found in the Frontier Province. The flies exist in large

numbers at Landi Kotal, at 3,500 feet, where the disease is met with in epidemic form during the hot weather period. At Cherat (4,500 feet) at a distance of 40 miles, and Abbottabad (4,000 feet) distance 110 miles, the fever is reported as occurring in sporadic form, suggesting that the cases are either imported from the plains, or else incorrectly diagnosed. In each of these places, a trained collector worked for two weeks, and the collections of flies brought down were despatched to Guindy for identification. No *P.papatasii* were among the specimens found at Cherat, and at Abbottabad only two were noted in the collection examined.

Examination of earth samples for sandfly's larvae has been carried out by means of a special apparatus, in which the soil is broken up in a concentrated solution of magnesium sulphate and the supernatant material floated off to a settling tank and thence to a filter paper on a Buchner funnel. Of fifteen specimens examined, two have been positive, both of which were taken from shady places and contained a fair amount of moisture.

Laboratory work.

Sera have been collected, during the first three days of fever, from seventy-eight cases of suspected sandfly fever. After dessication by a process of freezing *in vacuo*, over sulphuric acid, these sera were despatched to the King Institute for further examination. In Peshawar, seventy of these sera have been tested for the presence of virus by chorioallantoic membrane inoculation, with results as follows :—

Present 40, absent 16, presence uncertain 14.

An experiment was performed to investigate the effect of the dessicating process on bacteria contaminating serum. Suspensions of four organisms, which may be considered as possible laboratory contaminants, were kept in a frozen state (at —78° C) *in vacuo* over sulphuric acid for a period of 12 hours, and then transferred to a dessicator, where they remained *in vacuo* over the acid for a further three days. Three of the organisms, *i.e.* strains of *B.coli*, Flexner's dysentery bacillus (A 1) and *Staphylococcus albus* were still viable at the end of the process, and only one (*Streptococcus viridans*) failed to survive.

Fifty-one specimens of serum have been collected from sanfdly fever convalescent patients, at intervals of 40 to 180 days from the date of the onset of the fever, and are being despatched in an undried state to the King Institute for the invesitgation of neutralising antibodies. In an attempt to minimise the effects of external temperature on the five day journeys from Peshawar to Guindy, these sera are being packed in vacuum flasks.

The serial passages of the virus on egg membrane and in flask culture have been maintained. In the former the virus is at its 73rd passage and in the latter at its 67th passage.

The sera received from Peshawar were tested for the presence of virus in the Institute at Guindy. In the majority of sera tested at Guindy, the presence of virus could not be demonstrated. It appears the sera were subjected to abnormal temperatures during transit, which factor among others, probably accounted for this failure.

Serum and C. S. F. from a case of sandfly fever collected on the 1st and 2nd day of the disease respectively were tested for the presence of

virus. It was found to be present in both as judged by the lesions obtained on egg membranes. The lesions obtained with C. S. F. were also passaged successfully.

The C. S. F. from a case collected on the 3rd day of the disease did not show the presence of virus.

Attempts were made at Peshawar to isolate the virus from the urine of sandfly fever cases. Passageable lesion were obtained on egg membranes. The identity of the virus was not, however, finally determined by cross neutralisation tests.

Two batches of sandflies collected at Cherat and one batch from Abbottabad were submitted for identification. At Cherat, most of the specimens identified were *P.minutus*; while a few belonged to other species; no *P.papatasii* were identified. At Abbottabad, however, two specimens were identified as *P.papatasii*.

PUBLICATIONS.

One paper has been published and one submitted for publication embodying the results of this enquiry.

56. Endemic fluorosis enquiry under the Director, King Institute, Guindy.

The survey work carried out in selected areas of the Nellore District in 1938-39 indicated that, while the incidence and severity of chronic fluorine intoxication was related primarily to the concentration of fluorides in the water supplies, there were probably some other factors also in operation which tended to accelerate or aggravate the condition.

In the course of the general survey it was observed that in one Hindu village, Dirisavancha of Kanigiri taluk (2.8 p.p.m. fluorine content of water) the incidence of bone affections was almost negligible while its Madiga hamlet (3.2 p.p.m. fluorine in water) situated quite close to the main village showed a high and severe incidence, similar to that in Malapalli hamlet of Podili, referred to in last year's report to the Scientific Advisory Board. These two hamlets resembled each other but differed from the Hindu villages as regards the economic and social status of the respective inhabitants. It appeared possible, therefore, that nutrition might play an important role in producing the difference in the incidence observed in different villages. Accordingly, a nutritional and economic survey was initiated and the habits and dietaries of representative families in eight different villages and hamlets situated in the three different taluks were carefully studied and recorded. A fluoride-free village, Kaligiri, in the non-endemic coastal tract of the Nellore District served as the control. The technique of the dietetic survey was on the lines outlined in Health Bulletin No. 23. The surveys revealed the following important features :—

1. Even with a high fluoride content of the water used for drinking and cooking, the incidence and severity of chronic fluorine intoxication is greatly influenced for the better by an intake of nutrients which approximate to accepted international standards of sufficiency.

2. Even with a comparatively low fluorine content in the water used for domestic purposes, a deficiency in the intake of nutrients in diet results in a higher and a more severe incidence, even the younger adult age groups becoming affected. (cf. Kammavarpalli and Madigapalli of Dirisavancha).

3. In the absence of fluorides in drinking water (*e.g.* Kaligiri, a control hamlet) a deficiency in the intake of nutrients does not produce any symptoms of fluorosis such as mottled enamel, or bone manifestations.

4. Ragi is the cheapest of food grains and is the richest as regards calcium content. The Malas and Madigas as well as the poorer caste Hindus live almost entirely on ragi and obtain in their diet a much higher quantity of calcium than what is ordinarily required. Their vitamin C intake is very low, indeed almost negligible. The Madigas of Mittatmakur live mainly on cholum—with a calcium content equivalent to less than a tenth of ragi—and show a comparatively lower and milder incidence in spite of their using water with 2.2. p.p.m. of fluorides. The vitamin C content of their diets is higher than that of the Malas and Madigas of the other taluks surveyed, but is, however, still below standard.

5. Where the fluoride content of water is approximately the same in two or more villages (cf. Mittatmakur, Dirisavancha, Parvathipuram and Madigapalli of Kanigiri) the low incidence of the affection in Mittatmakur and Dirisavancha is found to be co-existent with a higher intake of vitamin C and a lower calcium intake as compared with other two hamlets, where a higher and more severe incidence is found to co-exist with a higher calcium and a lower vitamin C intake. Vitamin C and calcium being intimately associated with the development of bone and teeth, this observation becomes, therefore, all the more significant. The pronounced deficiency in the vitamin C content of the food intake probably interferes with the proper excretory functions and the excess of calcium ingested with the food serves to fix the fluoride in the system. There would, thus, appear to be a close correlation between the incidence of chronic fluorine intoxication and two of the main food factors in the dietary *viz.* calcium and vitamin C.

6. The nutritional status of the children is definitely *below par* in the whole area but the children of the poorer classes (Madigas) are definitely undernourished and show a greater all round deficiency than the caste Hindu children.

Reference was made in the last report to the effect of occupation on incidence and severity. The present survey confirmed that finding and showed in addition that the sites to be affected first were nearly always those in constant and regular use and that calcification and irregular depositions of bone occurred largely at points thus exposed to considerable stress and strain.

Three other factors, besides occupation and nutritional deficiencies were also found to aggravate the condition *viz.* (1) the meteorological factors *e.g.* high temperature, (2) the consumption of dirty water laden with

suspended impurities and (3) the culinary and dietetic habits of the people. The hot weather conditions in this arid region increase the intake of water by the residents and what is more, necessitate the use of silt and sediment-laden water owing to the scarcity of water in the wells. The grinding stone used for preparing food and condiments adds its quota of soluble and insoluble salts. The exact nature and extent of these additions and their role in the etiology of chronic fluorine intoxication have to be studied.

The results of the surveys so far carried out would thus appear to point unmistakably to the fact that the chronic endemic fluorosis in the Nellore District is atrributable to at least two outstanding factors, *viz.* the presence of fluorides in toxic doses in the domestic water supplies and a general state of chronic malnutrition with a pronounced C avitaminosis.

EXPERIMENTAL INVESTIGATIONS.

Laboratory experiments were undertaken to determine the extent, if any, to which dietetic factors noted in the survey influenced the onset and progress of chronic fluorine intoxication. Feeding experiments were started on monkeys. These were divided into three groups. Group A received a normal diet without fluorides and was not deprived of vitamin C. Group B received the same as above but with the addition of sodium fluoride 10 mg. per kilogram of body weight. Group C received a diet deprived entirely of vitamin C and had the same dose of fluoride as group B.

Skiagrams were taken of various bones and structures of one monkey from each of the 3 groups before the feeding experiments were started. Several biochemical examinations were made *e.g.* excretion of fluorine in urine and faeces, blood changes, etc., before and during the progress of the experiments. Skiagrams were again taken of the same monkeys 3½ months after the experiments were initiated.

From the investigations so far made, it is found that :—

Group A (control). has remained normal and showed no abnormal skeletal changes. These animals have also shown the normal increase in weight.

Group B (fluoride plus vitamin C). Skeletal changes suggestive of a mild affection, similar to those observed in human subjects, have been noted in the skiagrams. These animals have shown no appreciable decrease in weight but appear less active than those of group A.

Group C (fluoride and no vitamin C). As compared with group B, these animals have shown definite skeletal changes such as commencing ossification of interosseous membrane and new bone formation (periostitis). They have lost weight considerably and show marked limitation of movements analogous to those observed in human subjects in the affected areas.

These experiments are in progress and the detailed biochemical and other findings will be reported in due course.

The possible relationship of C avitaminosis to chronic fluorine intoxication as indicated in the record of the field survey would thus appear to receive confirmation from the data recorded above.

57. Snake venom enquiry under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

1. Enzymes in snake venom.

Besides those reported previously a number of other enzymes such as amino-peptidase, polypeptidase, carboxy polypeptidase, peroxidase, etc., have been detected in the venoms of cobra (*Naja Naja*), *Vipera ressellii*, *B. fascitus* and *Echis carinata*. Purification of choline-esterase detected in the venoms of *Naja Naja* and *B. fasciatus* by Ghosh and co-workers and by Chopra and co-workers has been attempted. After such investigation a method of separation has been evolved which consisted of saturation of a solution of corba venom with sodium chloride, collection of the precipitate and its solution in water followed by precipitation with sodium sulphate at 22% concentration, resolution of the precipitate in water and reprecipitation with sodium sulphate. In this way a sample was obtained which for the same weight showed ten times more choline-esterase activity than the crude venom.

2. The haemolysin in snake venom.

It was stated in last year's report that partial separation of the haemolysin from the neurotoxin has been effected in the case of cobra venom. Further work on this line has been done and as a result of this complete separation of haemolysin from the neurotoxin of cobra venom has been effected and the former has been obtained in a form which weight for weight is twelve times more active than the crude cobra venom. It has also been found that the action of haemolysin is similar to that of an enzyme and it contains a sulphydyl group in its molecule. The activity of haemolysin can be partly destroyed by oxidation and restored (provided the oxidation is not too drastic) by reduction. Partial separation of haemolysin from the neurotoxin in *rattle snake venom* has also been effected.

3. Separation of the active principles of Russell's viper venom.

(*a*) *The haemostatic or coagulating principle.* After much experimentation a method of partial separation of the coagulating principle from the neurotoxin and the haemorrhagin in the case of the venom of *Vipera rissellii* has been evolved. It consists of (1) precipitation from a one per cent solution of venom with anhydrous magnesium sulphate a 50 per cent concentration, (2) solution of the precipitate in water and reprecipitation with magnesium sulphate. This precipitate contained 90 per cent of the haemostatic principle along with small percentages of neurotoxin and haemorrhagin, (3) it is dissolved in water and further purified by fractional precipitation with anhydrous sodium sulphate. The final product

which we have obtained is, weight, for weight, about eight times more active than the crude venom. It, however, still contains about 6 per cent of neurotoxin and a trace of haemorrhagin.

(*b*) *The neurotoxin of Russell's viper venom.* Partial separation of the neurotoxin from the other constituents of Russell's viper venom has been effected by adopting the following procedure. A measured volume of one per cent solution of venom was treated with one-fourth of its weight of ammonium sulphate and filtered and the filtrate was again treated with the same quantity of ammonium sulphate as was used before. The precipitate obtained was dissolved in a measured volume of water and treated with twenty times its volume of saturated magnesium sulphate and filtered. This filtrate contains the major portion of the neurotoxin along with small quantities of haemorrhagin and the haemostatic principle.

4. THE ACTION OF CHEMICALS ON THE NEUROTOXIN OF *Naja Naja* VENOM.

The action of a number of compounds like sodium bisulphate, ascorbic acid, cysteine, hydrogen sulphide and zinc dust and dilute hydrochloric acid has been tried on the crude as well as on the purified neurotoxin of cobra venom. It has been found that *in vitro* they all reduce the activity of neurotoxin to a marked extent. The bisulphite is very active in as much as it destroys the neurotoxin in a few hours. So far all attempts to restore the activity of the inactivated neurotoxin have failed.

58. Enquiry into infantile cirrhosis of the liver under Dr. U. Srinivasa Rao at the Medical College, Madras.

The investigations for the current year were pursued in the following directions :—

(1) Collection of authentic clinical and etiological data, with a view to defining clearly the etiological and clinical complex of the disease, from (*a*) the general practitioners and specialists practising in the Province through the medium of questionnaires, (*b*) from a scrutiny of the hospital case-records and (*c*) from a study of Medical College autopsy protocols.
(2) Haematological study.
(3) Induction of cirrhosis in experimental animals with the object of observing the earlier stages in the pathogenesis.
(4) Study of portal circulation during intoxication with cirrhogenic agents in experimental animals.
(5) Further study of the morbid anatomy in infantile cirrhosis of the liver.

(1) COLLECTION OF CLINICAL AND ETIOLOGICAL DATA THROUGH QUESTIONNAIRES.

An attempt at collecting authoritative facts concerning etiological and clinical features of this disease was made by the distribution of specially

drawn up questionnaires to medical practitioners and service men, throughout the Presidency, through the District Medical Officers with the authority of the Surgeon-General. So far 765 questionnaires have been distributed to all the 24 District Medical organizations serving about 977 medical men. Through the Surgeon General, special contact is being established with medical specialists (62) working in different parts of the Presidency, towards the same purpose. So far 50 replies containing 90 answers have been received.

Analysis of the information already available from the answers to the questionnaire shows (1) incidence in age-groups varying from 7 months to 10 years with an average age of 2 years and 4 months; (2) racial distribution predominantly in the Hindu Brahmin community; (3) sex distribution ratio of male children to female children as 52 : 38; (4) 21.5% of the cases being breast-fed and the rest fed on cow's milk, rice etc.,; (5) clinical syndrome comprising hepatomegaly in all cases, splenomegaly in 74% of cases, clayey stools in 54% of cases, biliurea in 55% of cases, jaundice in 37% of cases and ascites in 30% of cases; (6) the incidence of the frdiseas is fairly universal in the Province, no district being exempt from this disease.

(b) COLLECTION OF CLINICAL AND ETIOLOGICAL DATA FROM A SCRUTINY OF THE HOSPITAL CASE RECORDS.

The clinical records of the General Hospital, Madras, from January 1928 to July 1939 have been scrutinized and the information available analysed and tabulated on lines similar to that outlined in the questionnaire. It is observed that attention to this condition as a separate clinical entity in hepatic disorders of children appears to have been accorded in the hospital records only in recent years. A large majority of the 60 case-records available, furnish meagre etiological and clinical data regarding this disease.

From among a total of 340,000 case records examined 60 cases of infantile cirrhosis were encountered. The clinical and etiological data available is summarised below :—(1) The age incidence varied from 1½ months to 10 years, the average age being 3 years and 7 months; (2) the sex ratio of male children to female children was 35 : 25; (3) racial incidence was predominantly among Hindus, 2 cases also being in Indian Christians; (4) the main features of the clinical syndrome were ascites in 82% of the cases, hepatomegaly in 67% of cases, splenomegaly in 55% of cases, jaundice in 37% of cases, and biliurea in 16% of cases.

(c) COLLECTION OF CLINICAL AND ETIOLOGICAL DATA FROM A STUDY OF THE MEDICAL COLLEGE AUTOPSY PROTOCOLS FOR 10 YEARS.

No case came up for autopsy during the year under report. During the above period only four autopsies on children dying from infantile cirrhosis were conducted.

(2) HAEMATOLOGICAL STUDY.

Haematological investigation of all available cases (18) was carried out to enable the establishment of definite haematological standards for this disease. On account of the difficulty experienced in obtaining a sufficient quantity of blood from the emaciated children referred mostly

in a condition of "in extremis", we had to be content with only a limited number of haematological investigations in each instance. In a few instances it was possible to make more detailed investigation.

The averages of the results obtained are as follows :—

(1) Haemoglobin 54.1% ; (2) Mean corpuscular haemoglobin content 13.70 micro-micrograms ; (3) Mean corpuscular haemoglobin concentration ; 34.71 grms. per 100 cc. of packed cells ; (4) Total R. B. C. : 343,7671 per cmm ; (5) Mean average diameter of the red cell 7.31m ; (6) Mean corpuscular volume ; 93.5 cubic microns ; (7) Volume index 0.93 ; (8) Sedimentation rate 3.05 (Wintrobe's method) ; (9) Colour index 0.76 ; (10) Total W. B. C. 14,200 per cmm. ; (11) Differential count ; Polymorphs 57.8%, Lymphocytes 35.8% ; (12) Schilling index ; myelocytes 5.8%; juveniles 4.8%; stabs 8.8%; filamented 80.6% ; (13) Platelet count ; 261,377 per cmm. ; (14) Reticulocytes 1.92% ; (15) Saturation index 0.95 ; (16) Gel test positive in 8 cases ; (17) Vanden Bergh reaction ; direct positive in 3 cases out of 7 and indirect positive in 2 cases out of 7.

(3) Induction of cirrhosis in experimental animals with the object of observing the earlier stages of the pathogenesis.

An extension of this work in experimental animals of earlier age-groups appeared justified. Besides, the morbid anatomical study of the earlier stages of the disease can only be done in material from experimental animals. Hence experimental induction of toxic cirrhosis in a litter of pups 1½ months old by the administration of carbon tetrachloride was resorted to.

A litter of 6 pups was first selected one of which served as the control. Of the remaining, 3 received per orum carbon tetrachloride suspended in milk in increasing doses (1-23 minims B.D.). The other two received the drug parenterally (1-5 minims) every third day. So far five animals died. The morbid anatomical study of the autopsy material from these animals is under progress. Even though the microscopic appearances so far observed suggested the presence of slight sclerotic changes in the hepatic veins even in these eraly cases, the gross study by means of corrosion preparations did not reveal any marked changes in the organ. Hence the induction in animals in which artificial production of toxic cirrhosis has been already successfully accomplished, namely the albino rats, was subsequently resorted to. In a group of 6 albino rats, 3 are receiving 0.2 c.c. of carbon tetrachloride once a week and the rest 0.2 c.c. twice a week subcutaneously. This experiment is under progress.

(4) Study of portal circulation during intoxication with cirrhogenic agents in experimental animals.

(*a*) *In isolated livers.* The exact pathogenisis of the pre-cirrhotic central necrosis in toxic and infantile cirrhosis was suggested by P. R. Rao to be vascular (ischaemic) in nature and probably resulting from a vaso-motor inbalance (vaso-spasm) induced by the toxin. M. V. R. Rao however did not feel justified in supporting such a theory. The present experiment is devised to throw light on the mechanism leading to the central necrosis.

Isolated, canine livers were perfused at a constant rate and pressure in a Dixon's apparatus with Locke's fluid containing defibrinated, oxygenated blood. The delivery from the hepatic vein per minute was measured. After a number of preliminary observations carbon tetrachloride in gradually increasing doses was introduced into the portal canula periodically and the variations in the outflow recorded. Seven experiments were done and in all a transient diminution in flow suggesting a vaso-constrictor effect was observed. In 3 instances after the introduction of 2.5 c.c. of the drug this effect failed to appear suggesting a fatigue phenomenon. In one instance this change appeared after 1.5 c.c. and in another after 6 c.c. of carbon tetrachloride.

(*b*) *In livers in situ.* Eight attempts made in exteriorising the hepatic circulation with a view to studying the action of cirrogenic toxins on the portal circulation with the liver in situ, were not successful. Attempts at perfecting this technique are being continued.

From the results obtained with isolated livers it is evident that if the diminution of the outflow was due to toxic damage of the parenchyma and consequent mechanical obstruction to the circulation, the effect would not have tended to disappear with the progress of the experiment, as it did. The transient nature of the change and the appreciable pallor of the organ after each injection would suggest a vascular (vaso-motor) origin for the curtailment of the circulating fluid immediately after the introduction of the drug into the portal canula.

(5) FURTHER STUDY OF THE MORBID ANATOMY IN INFANTILE CIRRHOSIS OF THE LIVER.

(*a*) *Naked eye and microscopic study of the condition of the vascular trees in the liver in infantile cirrhosis.* Both gross and microscopic appearances of the liver reveal a progressive curtailment of the branches of the hepatic venous tree, with fibrosis in the regions served by such occluded branches. The work summarised below was an endeavour to establish an exact, gross anatomical and microscopic confirmation of the above findings. The sectioned surface of specimens of cirrhotic livers cut longitudinally according to plan was subjected to a systematic enumeration of the cut ends of the vessels recognizable with the aid of a hand lens (magnification X $2\frac{1}{2}$). Stained paraffin sections of the organ removed from the right and left lobes according to plan were similarly subjected to enumeration per sq. cm. under low magnification (1/3), of the branches of the portal and hepatic veins with the aid of a 5 mm. sq. eye-piece micrometer interposed in the eye-piece.

The enumeration by the gross method (hand lens) revealed a ratio of 1 ; 1.5 between the recognizable segments of the hepatic and portal veins respectively and an inversion of this ratio in the case of infantile cirrhosis. Microscopically the ratio was unit in the case of the normal liver and 1 : 3 in infantile cirrhotic livers.

The result affords a quantitative confirmation of the findings, namely, sclerosis and destruction of the branches of the hepatic venous tree in infantile cirrhosis.

(*b*) *Microscopic anatomical study.* The microscopic anatomical study of 7 specimens carried out is mostly confirmatory of the findings published by M. V. R. Rao. Evidence in support of the distortion and mutilation of the hepatic venous tree in toxic cirrhosis, with the help of radiograms and corrosion preparations, has been added.

59. Fly research enquiry under Dr. D. N. Roy at the School of Tropical Medicine, Calcutta.

Attention has been mainly confined to the collection of house-frequenting flies from different localities with a view to studying their habits in nature. Until now more than one thousand flies of all species have been collected.

The large amount of data collected during the last year by Mr. Siddons on the seasonal breeding habits of flies is being analysed.

The genetical problem with the species of *M. domestica, M. vicina* and *M. nebulo* has been taken on hand.

Studies on the life history of a Chalcidoidea, parasite of fly pupa, are nearly complete.

60. Filariasis enquiry under Dr. T. Bhaskara Menon at Vizagapatam.

Artificial cultivation of *Mf. bancrofti* was studied. Blood containing a large number of embryos was repeatedly inoculated into nutritive as well as non-nutritive media with a view to determining the cultural behaviour of the embryo. The media employed were non-heated medium of Row, also after the addition of glucose, Ringer-Locke solution, also with addition of human plasma, glucose broth, peptone digest medium of Harley, sterile .85% saline and whole egg. Current bacteriological technique was employed with the media in small test tubes. The result of this study may be summarised as follows : (*a*) *Mf. bancrofti* could be kept alive 'in vitro' for a period of about fifty days, especially when maintained at a temperature of about 10° C. The most favourable medium was normal saline. (*b*) Contrary to the results reported on the culture of the embryo of *Dirofilaria immitis*, the filaria of dog, no growth or change of morphology could be observed with *Mf. bancrofti* even at the end of fifty days. (*c*) Many of the embryos were dead after the first two weeks and those that were alive were very sluggish. (*d*) Many embryos lost their sheaths in a few days in such cultures and this led to the investigation of the ex-sheathing factor.

The factor which is responsible for the ex-sheathing of the embryo in the foregut of the mosquito was investigated. Many factors such as pH. variation, digestion alteration of the viscosity and temperature of the surrounding medium were supposed to effect this and the influence of each of these on the sheath of the embryo was studied systematically in a series of experiments 'in vitro'. The range of pH. variation studied was from 4.1 to 10 consisting of about thirteen solutions in series. Pepsin was employed to obtain artificial digestion, fibrin for increase of viscosity and chilling and thawing for temperature variation. It was observed that none of these factors had any influence on the sheath of the embryo. The observation that many *Mf. bancrofti* lost their sheaths in cultures, however, led to the investigation of the factor responsible for this phenomenon. It was found out by constant observation that large numbers of embryo were entangled in fibrin-leucocyte masses that tended to form in cultures and were derived from the inoculated blood. This caused an anchoring of the sheath and was followed by a gradual penetration of the sheath by the embryo. The embryos were concentrated in those masses in such numbers as to suggest some form of taxis. This process of coming out of sheath took from 4 to 24 hours. This observation was confirmed by a study of the natural ex-sheathing in the mosquito foregut. When a mosquito fed on filarial blood was dissected 6 to 12 hours later, the same process of wriggling out of sheaths caught in fibrin-leucocyte masses could be seen.

The biology of the infective larvae and the possibility of experimental transmission to laboratory animals are being studied.

61. Anthropological study of Indian brains under Mr. R. Krishna Rau at the Medical College, Vizagapatam.

Whereas extensive studies and observations have been made by anthropologists in the west on European, African, Australian, Chinese, Japanese and other brains, negligible and insignificant have been the contributions to the literature on Indian brains. Only one Goanese (Indian ?).brain has been described by Cole and two brains, one of a Madrasee and the other of an Andhra, have been studied in detail by Appleton at Cambridge. The aim and object of this paper are a detailed macroscopic study of the brains of the Telugu speaking people of the Vizagapatam District with a view to determining their distinctive characters by a comparative study.

Material.

The material of this paper comprises a collection of twenty-eight brains obtained from the cadavers received in the Department of Anatomy, Medical College, Vizagapatam. Out of them, twenty have been studied in detail till now. They are of the proletariat classes, peasants and workers eking out a precarious living with the sweat of their brow. They belong mainly to the district of Vizagapatam. All of them are Hindus belonging to the fourth caste, Sudra, and to the agricultural tribes or communities designated as Gavaras, Gollas, Kapus and Kammas.

Sex.

Of the twenty-eight unselected brains, 16 are of males and only 4 are of females and the remaining 8 of adults of unknown sex and age. It must be confessed that accurate information on the age of the subjects is not available in the department. The rustic rural folk never seem to bother about their age. All that can be said of the age of the subjects is that the brains are of adults ranging between thirty and fifty years of age. The technique of embalming of cadavers is the usual injection of a strong solution of arsenious acid in bicarbonate of soda. This is followed by a second injection of four ounces of pure formalin in the crania through the superior orbital fissures with a view to hardening the brains still further. Whole brains are removed and carefully transferred to cylindrical jars filled with preservative fluid (10% liquor formaldehyde) with base uppermost.

General configuration.

It is presumed that the outline of brains roughly corresponds to that of the skulls. Sergi classifies the skulls under five groups, ovoid or ovate, rectangular with the right angles rounded off, pentagonal, ellipsoidal and nearly circular.

The modal outline of the eighty skulls studied in this department may be described as an ovoid with the narrow pole in front and the broad pole behind, the broadest part being the biparietal diameter in some and bisquamosal diameter in others. The percentage of skulls falling under this category is 57.5%. This description may be said to apply to the brains under reference. (R. Krishna Rau and B. S. Guha. Qualitative and quantitative study of the crania of the Telugu speaking people of the Northern Circars). The predominant head form noted by a somatometric survey of the students of the Medical College, Vizagapatam, is mesaticephalic with an average of 76. It has been found that the average length —breadth index of the brains is 74. The typical or modal brain is dolichencephalic.

General features of the brain as a whole.

The main contour as has been remarked before is roughly ovoid. The method adopted for the skulls of various normae is also deemed appropriate for the study of the configuration of brains as a whole. There is a variable degree of asymmetry, slight in some and pronounced in others, of the two cerebellar hemispheres with respect to the size as determined by measurements, contour and sulcal pattern. No attempt has been made to study the cerebellum in detail in this communication. All the observations relate to the cerebral hemispheres.

Norma Frontalis.

When viewed from the front it is possible to see as far back as the post-central gyrus. The frontal pole is as a rule fuller and more rounded than the occipital pole ; the rostrum or the orbital keel is slight. The juxtra-rostal concavity is not pronounced. The pre-central frontal area is narrower than the post-central parietal region.

Norma lateralis.

The general disposition of the gyri is longitudinal, except the central and post-central gyri. The area of maximum width of the brain as a whole

corresponds to the inferior parietal lobule, which is as a rule well developed and complex. In 24 out of 36 hemispheres, there is no exposure of the insula; in 12 hemispheres, it is slight and negligible, the maximum triangular area of exposure never exceeding 12×4 mm. or 24 sq. mm. It may be mentioned that a small area will become exposed in all brains when the leptomeninges with the big sized middle cerebral artery and its numerous branches are stripped and removed from the depths of the insula. As compared with temporal lobes the parietal lobes are fuller and more bulging than the former. The prominence caused by the cap of Broca is variable.

Norma verticalis.

When the entire brain including the cerebellum is placed flat on the table the summit of the convexity of the supero-mesial border corresponds to the junction of the sensory-motor area, the upper part of the post-central gyrus. There is an area of flattening in some hemispheres in the region behind the parieto-occipital fissure. In most cases the temporal lobes are concealed from view when looked at from above.

Norma occipitalis.

The cerebellar hemispheres are completely concealed from view in all cases and overlapped by the occipital lobes. The occipital poles are narrower and more pointed than the frontal poles. There is no steepness of the occipital area. The superior longitudinal sinus grooves the right occipital pole in 21 brains, (75%) the left occipital pole in 4, and both the right and left poles in 3 cases, in a total number of 28 brains. The sulcus lunatus can be made out only in 17 out of 56 hemispheres. The sulcus lunatus in one or more parts is found more often on the left side (in 10 brains) and to a lesser extent (in 7) on the right side. Even in cases where the sulcus has been met with, it does not conform to the type found in simians.

Norma basilaris.

No keel (Syn. Olfactorius-wulst, orbital operculum of Waldeyer) of the pointed beak-like type figured in photographs and casts of prehistoric and the so-called primitive brains has been met with in any brain of the series described. The temporal poles are narrow and somewhat pointed in some hemispheres. "Uncus Deckel", inturning of the poles is as a rule absent though it is faintly seen in a few brains. The petrosal impression caused by the eminentia arcuata on the anterior surface of the petrous pyramid is present in 11 hemispheres out of 56 and faint or illmarked in 17 hemispheres. The post-orbital limbus described by Spitzka is more often present and is absent only in a few hemispheres. This is perhaps due to compensatory overgrowth of the orbital surface of the frontal lobe backwards beyond the sylvian border of the orbito-sphenoids towards the middle fossa of the base of the skull. Its incidence is fairly frequent. The opto-peduncular fossa (fossa Tarini) is narrow. The medial olfactory tract is most often indistinct; the tubercle is faint and inconspicuous. The concavity of the tentorial surface caused by the upper part of the cerebellar hemispheres is fairly well seen in all hemispheres. Pre-occipital notch caused by the fold of dura over the parieto-mastoid suture or by the posterior anastomotic vein of Labbe, is variable.

Weight of the brains.

It may, however, be remarked that the weight of the brain depends not merely on the complicated pattern of the brain but also on the size

and stature of the body as a whole. The standard technique prescribed by Ariens Kappers for weighing the brain is that the brain should be completely stripped and that the brain stem should be out at the level of the calamus scriptorius and that the ventricles should be emptied, the fluid contents of which, according to Harvey weigh 34 grams. The method followed by the writers is that the brains divested of the membranes were weighed after gently mopping them with a dry towel. No special attempt was made to empty the ventricles by pressure. The series selected for recording the brain weights consists of 15 brains of male adults and 4 brains of adult females. The average brain weight of the 15 adult male brains is 1208 grms. and that of the four adult females is 1027 grms. The weights taken are :—

1. The weight of a brain as a whole,
2. Weights of the individual hemispheres including fore and mid-brains,
3. Weights of right and left halves of hind brain.

COEFFICIENT OF CEPHALIZATION.

The coefficient of cephalization is a generally accepted expression of the "cognitive ability" of the brain as distinct from the moiety which pertains to the sensory and motor supply of the body. It is given by Dubois by the formula $K=E/P^r$ (where K=coefficient of cephalization, E=brain weight in grms, P=body weight in grms and r=0.56 relation exponent).

To calculate the coefficient of cephalization it is necessary to know the brain weight and body weight.

Kappers suggests that to get the corrected brain weight one per cent should be subtracted from the formalin hardened brain weight. Applying this correction, the corrected brain weight for males in this series might be given as 1196 grms.

As regards the average body weight of the healthy adult here, we had no previous figures of other workers to proceed on. So it became necessary to work out as a side investigation the average body weight for the male adult in the locality. The weights and heights of seventy-five people, servants working in our institution, were measured. A height frequency curve was plotted and the modal height was noted and the average weight of people belonging to this peak-group was calculated. It is 107.5 lbs=48,762 grms.

Applying the formula for the coefficient of cephalization to our data, taking brain weight as 1196 grms and body weight as 48,762 grms, K is found to be 2.834.

For purposes of comparison it might be mentioned that the coefficient of cephalization in European males according to Kappers is 2.74. The coefficient of cephalization in South Indian brains is therefore definitely higher than that in European brains.

62. Peptic ulcer enquiry under Major J. R. Dogra, I.M.S. at the King Institute, Guindy, Madras.

The work of this enquiry, started early in 1938, was continued during this year. It mainly consisted of :—

(a) Clinical studies.

The clinical studies were confined to proved cases of the disease. Such cases, now 265 in number, have been studied at the Government General Hospital, Madras, with special reference to pathological findings at operation, clinical signs and symptoms, diet, habit, economic state and other environmental conditions of the patients. This study, made with particular reference to the pathological process involved, has resulted in the clarification of the clinical picture of the disease as encountered in Madras. Briefly, it may be stated that the majority of cases were chronic ones of long duration and came almost exclusively from a very low social status, where malnutrition and extreme poverty were outstanding features. Of 258 cases, there were 4 who had gastric ulcers, in 11 duodenal and pyloric ulcers occurred together and in 243 duodenal ulcers alone were present.

Pathological studies revealed the disease to be a progressive process. The early cases showed an inflammatory lesion in the first part of the duodenum, chiefly involving its anterior wall, with no pyloric obstruction or organic stenosis and no marked changes in the stomach. The advanced cases showed varying degrees of pyloric obstruction and in a very large number of cases, organic stenosis and marked changes in the size, shape and mucosa of the stomach with the corresponding signs and symptoms.

Preliminary investigations at Vizagapatam and various centres on the West coast (Neyyoor, Trivandrum, Quilon, Kottayam, Alleppey, and Ernakulam) consisting of consultations with physicians and surgeons at each centre along with study of cases and hospital records revealed that, although climat cally, environmentally and in many other respects the West coast differs greatly from the East coast, yet the essential features of peptic ulcer regarding the pathological process, clinical features and economic and social state of those who suffer from this disease were similar to those seen in Madras.

(b) Statistical.

For the purpose of statistical evaluation, an extensive study of data has been undertaken. Hospital records at Vizagaptam, Madras, and Neyyoor are being studied. In order to obtain accurate data to ascertain the extent and prevalence of the disease in different parts of the province, co-operation of the provincial medical organisation has been enlisted. A special proforma has been drawn up and circulated to all the District Medical Officers. With the assistance of Major-General N. M. Wilson, I.M.S., Surgeon-General with the Government of Madras, it is hoped that data will be available from all over the province. A similar procedure has been adopted by the states of Travancore and Cochin on request. The Director-General, Indian Medical Service, has also been approached to enable collection of similar data from other provinces in India.

(c) Experimental.

Experimental production of peptic ulcers in monkeys, which was started in the later months of 1938, was continued to study the effects of varying diet factors in relation to peptic ulcer production. The gastric response to the food factors in animals with reference to acidity is being studied.

(d) Pathological.

Detailed histological investigation of biopsy and post-mortem material was continued to study the pathological features of peptic ulcer in South India. Attention was recently drawn to the changes in Aeurbach's plexus in ulcer cases by Drs. Orr and Rao. This problem is under further study. Gastric mucous membrane from cases of peptic ulcer has been examined with a view to studying the role of gastritis in the causation of ulcer.

REPORTS OF ADVISORY COMMITTEES.

REPORT OF THE CHOLERA ADVISORY COMMITTEE.

The committee met at 10 a.m. on December 18th 1939 and considered the reports of the different enquiries.

THE BENGAL FIELD CHOLERA ENQUIRY UNDER DR. S. R. PANDIT.

The area selected for the enquiry was the Khulna District which is considered to be an endemic area. Two areas were selected for investigation which had known heavy incidence of cholera and presented varying conditions of salinity of waters. A small epidemic was investigated in July which was due to the Ogawa sub-type of *V.cholerae* and this type was also found in contacts and in waters in the area. In the absence of epidemics, systematic bacteriological examination of water samples and stools by different methods for the isolation of vibrios was carried out and chemical examination of waters for total solids, salinity, and organic matter content, in relation to the bacteriological findings. In both areas agglutinable vibrios of both the Inaba and Ogawa sub-types, haemolytic and non-haemolytic, were isolated. The prevailing type was Ogawa haemolytic. The percentage of isolations decreased with decreasing salinity of the waters. Both haemolytic and non-haemolytic strains were isolated at the same time in some cases and considerable variations in haemolytic power were noted in the case of several strains. Many strains were sent to Kasauli for detailed study of serological and haemolytic properties. Non-agglutinable vibrios were isolated from stools in the absence of cholera cases but as the stools often showed a reaction definitely on the acid side it is unlikely that they would have survived, if present. The salinity of waters in the area decreased with the onset of the monsoon. A high salinity was associated with low total solids/chloride ratio and low salinity with a high ratio. The organic matter content of most samples was adequate to allow of multiplication of vibrios. Of 631 water samples from the area of high salinity vibrios were isolated from 6.3 per cent and of 397 from the area of low salinity 2.8 per cent were positive.

Out of 125 blood samples taken from individuals from the Khulna District 8 agglutinated Inaba or Ogawa suspensions to 1/25 to 1/50.

THE MADRAS CHOLERA ENQUIRY UNDER THE DIRECTOR, KING INSTITUTE, GUINDY.

The headquarters of this enquiry was established at Tanjore in the middle of May 1939. Up to date there has been no cholera in this or the neighbouring districts and investigations had to be limited to the study of waters and to stools of persons using them. This was done in two selected areas which in previous years had been amongst the first to report cases of cholera and had experienced severe epidemics. A total of 145 water specimens was examined by the use of the mannose bismuth sulphite medium and an agglutinable vibrio was isolated on one occasion only, the source being negative when re-examined two days later. Mannose-fermenting inagglutinable strains were isolated from practically all tanks and river waters examined and in their presence the medium employed is not capable of yielding differential isolation of *V.cholerae*.

Estimations were made of total chlorides and oxidisable solids, organic matter of the waters and in general it was considered that the composition of most waters was not suitable for prolonged survival of *V.cholerae.*

Out of 1488 stool examinations done none showed *V.cholerae* while inagglutinable vibrios were isolated from 57.

Observations were made on the period of survival of *V.cholerae* in water under laboratory conditions. With a minimal inoculum survival up to 74 days was noted in 2% sodium chloride and 54 days in 1% sodium chloride. In 3% sodium chloride recovery was not effected after 32 days.

CHOLERA VIBRIO ENQUIRY UNDER THE DIRECTOR, CENTRAL RESEARCH INSTITUTE, KASAULI.

The work undertaken was mainly the detailed examination of strains for the Bengal field enquiry. A large number of strains from waters and stools was examined, the majority being Ogawa sub-type and haemolytic. Both haemolytic and non-haemolytic were in some cases obtained from the same source and variations in the degree of haemolysis produced from time to time by the same strains were noted. A method for quantitative estimation of degree of haemolysis was developed, including the use of a fixed reproducible standard solution of acid haematin with which comparisons could be made. The presence of these El Tor strains in the Khulna area of Bengal is a phenomenon not previously observed in India and the variability of their haemolytic action presents an important subject for study in relation to the general problem. A number of inagglutinable strains from the same course was also examined and typed as far as possible.

Studies were made in haemolysin production under different conditions and also on methods for the differentiation of El Tor and cholera vibrios.

Although not included in the original programme for the enquiry, methods applicable to the testing of cholera vaccine were investigated, including the study of the changes which may be expected in a vaccine on prolonged keeping.

THE CHOLERA STATISTICAL ENQUIRY UNDER DR. R. B. LAL.

The work on the classification of the districts of South West Bengal in regard to their endemicity and epidemicity was continued. The method elaborated for forecasting cholera in Calcutta was applied to other homogeneous districts and found applicable to 10 out of 19 districts. An analysis was undertaken of the field data collected during the investigations in the Diamond Harbour area to determine the possible correlations existing among the factors which describe the population of the area. The factors, studied were relationship of health with age, occupation, economic status, appetite bowel movement and diet. It is hoped to complete the analysis during the year.

CHOLERA BACTERIOLOGICAL ENQUIRY AT THE SCHOOL OF TROPICAL MEDICINE, CALCUTTA, UNDER MAJOR C. L. PASRICHA, I.M.S.

Study of cholera cases.

In the first half of the year 173 cases of clinical cholera were examined bacteriologically by the use of peptone water enrichment and by enrichment in the modified Wilson and Blair medium followed by plating on

Aronson's medium and also by the use of bile-salt agar. The modified Wilson and Blair medium gave the best results.

The detailed serological examination of 959 strains isolated from cholera cases in Calcutta showed 60.7% to be of Inaba sub-type and 25.8% of Ogawa sub-type. In the series 10.7% of vibrios were non-agglutinators. Differences were found in the results of agglutination tests with the O sera when formalised or boiled suspensions were used, the latter being less reliable.

Cholera vaccine.

Samples of cholera vaccine from different sources were tested in regard to sterility and potency in relation to circular No. 1 of 1939 from the Central Advisory Board of Health.

Chemical studies.

Studies were made in the blood of 17 cholera cases. It was found that (*i*) fibrinogen is markedly increased, (*ii*) there is an increase in blood urea, sugar, inorganic phosphate, total proteins, globulins and a relative decrease in chlorides. These findings are similar to that occurring in secondary shock and explain the effect of intravenous saline injections.

Bacteriophage studies.

The type of cholera phage in the stools of cholera cases was investigated and only type A was found.

Agglutinable vibrios in natural water.

Agglutinable vibrios were isolated from 4 out of 77 water samples examined from areas in which cholera cases had occurred and where there was evidence of recent contamination. The successful isolations were on the modified Wilson and Blair's medium, other media giving negative results.

Agglutinable sera.

The domestic fowl was found suitable for raising high titre sera and satisfactory titres were obtained with the dried O antigens. Fowls have many advantages for this purpose and are useful in testing cholera vaccines.

THE CHOLERA TOXIN AND IMMUNITY ENQUIRY UNDER DR. H. GHOSH.

The growth of *V.cholerae* was studied in different media in relation to pH and the presence of sugars. The presence of 0.2% glucose or maltose resulted in a rapid fall of pH and death of the vibrio. The addition of buffer salts such as di-sodium hydrogen phosphate did not prevent this. With the addition of 2% calcium carbonate to broth of pH 8.6 containing 0.3% maltose and 0.1% glucose good growth was obtained and the pH rose to 9.1 within 8 to 9 days.

Broth containing sodium acetate and di-sodium hydrogen phosphate but without a sugar yielded a filtrate, 0.25 cc. of which killed guinea-pigs in 24 hours. The toxicity of the filtrate was not altered by heating at 100°C for one hour. The toxic affects and the post-mortem changes occurring were studied. These experiments were carried out with an Inaba type strain. Filtrates from other strains including inagglutinable vibrios were being studied.

CHOLERA KIDNEY ENQUIRY UNDER DR. D. N. BANERJEE.

The changes in the kidneys of 26 fatal cholera cases were studied in which removal of the organ had taken place within half an hour of death. Degenerative changes were noted in the tubules and glomeruli, special staining methods for the basement membrane being employed for the studies. Gross damage to the endothelial lining of the blood vessels was observed.

Observations were made on the functioning kidney of the frog by perfusion experiments with vital dyes. The passage of neutral red and janus green through tubules and glomeruli was recorded. Histological studies on the permeability of the endothelial lining of the capillaries in cholera were also undertaken.

Observations were made on blood concentration with retention of waste products and on loss of chlorides.

Recommendations on the proposals for the continuation of enquiries during 1940-41.

BENGAL CHOLERA FIELD ENQUIRY.

It is recommended that this enquiry be continued on the basis of the proposals put up and that a grant of Rs. 27,000/- be given for this purpose. The headquarters of the enquiry will be established in Calcutta and investigations carried out in contrasting selected areas in the field. It is considered that, in the event of developments in regard to occurrence of cholera in the area now under investigation, indicating the advisibility of continuing work in the Khulna district beyond March 1940, it should be left to the discretion of the officer in charge of the enquiry to prolong his work in the area for such period as may be necessary.

CHOLERA ENQUIRY UNDER THE DIRECTOR, KING INSTITUTE, GUINDY, MADRAS.

It is recommended that this enquiry should be continued and that a grant of Rs. 8,795/- be given. It is noted that, under present conditions, the King Institute, Guindy, may not be able to furnish assistance previously given and that some increase over the previous year's budget may be necessary. The lines of work to be carried out should be in continuation of those at present in progress.

CHOLERA VIBRIO ENQUIRY UNDER THE DIRECTOR, CENTRAL RESEARCH INSTITUTE, KASAULI.

A grant of Rs. 300/- is recommended for this enquiry, which will be ancillary to the Bengal field enquiry and will carry out for it the preparation of agglutinating sera and special study of vibrio strains and related work.

CHOLERA STATISTICAL ENQUIRY UNDER DR. R. B. LAL.

It is not proposed to continue this enquiry during 1940-41.

CHOLERA BACTERIOLOGICAL ENQUIRY UNDER MAJOR C. L. PASRICHA.

In view of the new lines of work which have been taken up and are proposed to be continued by the enquiry, specially in relation to

the selection of straisn for cholera vaccines, the testing of vaccines etc., the continuation of this enquiry is recommended. As the Bengal field enquiry will also be located in Calcutta it is considered that co-operation between the laboratory and the field enquiry will be of value. A grant of Rs. 6,800/- is recommended for the enquiry under Major Pasricha.

CHOLERA TOXIN ENQUIRY UNDER DR. H. GHOSH.

A grant of Rs. 616/- is recommended for the continuation of this enquiry for a period of two months only, after which the enquiry will be closed down.

CHOLERA KIDNEY ENQUIRY UNDER DR. D. N. BANNERJEE.

In view of the accepted policy of the Cholera Advisory Committee that the resources available for cholera research should be devoted mainly to field work the continuation of the grant for this enquiry is not recommended under present conditions.

No proposals were received for new enquiries.

This committee note that the experience of certain members during the last year further indicates the necessity for the examination of cholera vaccines obtained from different sources as to their preparation from suitable vibrio strains as recommended by the committee in December, 1938, and by the Central Advisory Board of Health.

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 2 p.m. on 18th December, 1939, and considered the reports dealing with research work on malaria carried out during the current year and the proposals for work during the ensuing year.

(i) MALARIA INSTITUTE OF INDIA.

The Director amplified the report previously circulated on the work of the Institute, and laid special emphasis on certain features.

A number of tours have been undertaken by officers of the Institute in connection with the rural schemes which are in progress in various provinces, and to inspect the work of the field research units of the Institute.

After touring in Southern India in May, Lt.-Col. Covell went on to Singapore to lecture at the Instructional Course on Malariology for medical officers and engineers held under the auspices of the League of Nations. On his way through Ceylon he took the opportunity of seeing something of the anti-malaria work which is being carried on there by the Ross Institute and Ceylon Government. In Malaya he visited a number of localities where anti-malaria work is in progress and on the return journey he was able to visit Rangoon and discuss the malaria problems of Burma with the health authorities there. Dr. Puri visited Karachi from 24th April

to 9th May to carry out tests on the efficacy of various insecticides in spraying aircraft.

A conference of Chief Medical Officers of Railways took place at Delhi in the spring and Lt.- Col. Covell took this opportunity of discussing railway malaria problems with the officers concerned. The members of the annual conference of Irrigation Engineers visited the field station of the Institute in Delhi in November, when interesting discussions took place regarding the effect of irrigation on malaria. Lt.-Col. Covell also attended a meeting of the Botanical and Physiological Committee of the Advisory Board of the Imperial Council of Agricultural Research, when the questions of pyrethrum and cinchona cultivation in India were discussed.

The annual malaria course for medical officers was held this year at Delhi for the first time. The number of students was increased to 24 in conformity with the resolution passed by the Medical Research Conference of 1938.

A new edition of the practical entomological course for medical officers has been published in the health bulletin series of the Government of India. Revised editions of two others are in the press, as is also a new bulletin entitled 'Lectures on Malaria'. There are now 13 of these bulletins, and taken together they represent a text-book on malaria of more than 1,000 pages, which is constantly being brought up to date by the issue of new editions. There has been a continued demand for the chart illustrating the action of quinine and synthetic drugs on malaria which was published last year. Cyclostyled notes on various methods of malaria control have been widely circulated.

Scientific material has again been sent to many workers both in India and abroad, and the routine entomological work of the Insitute has been carried on as in former years.

A number of insecticides have been tested, including samples of pyrethrum flowers grown in Kashmir and in the Nilgiri Hills. Encouraging results were obtained from samples received from both these localities. In Kashmir the Forest Department has produced enough seed to bring 5,000 acres of land under cultivation next year. If these flowers come up to the standard of the sample received from the Agricultural Department of Kashmir State, it appears India will be able to produce sufficient pyrethrum for her needs at no distant date. It is essential, however, that the cultivation of pyrethrum in this country should be under control, as there are many points which must be scrupulously observed in order to produce an effective insecticide, as for instance the exact time for harvesting the crop.

The antimalaria operations in Delhi have again been supervised by officers of the Institute, the methods employed being similar to those practised in previous years. The control of malaria has been very complete, but it is recognised that the malaria department has been once more greatly aided by deficiency of monsoon rainfall.

The transfer of the field station from Karnal to Delhi was completed early in the year and has greatly facilitated the working of the organisation.

As regards the field researches, in Delhi observations have been made on the bionomics of *A.culicifacies* and the data collected is being analysed for publication. Further observations have been carried out regarding the spraying of pyrethrum insecticides in villages, and in the

development of oil booms for dealing with larval drift. In the Wynaad, further experiments have been made with the method of herbage packing to control the breeding of *A.fluviatilis* with encouraging results. A paper embodying the results of the work in this area is now in the press. In the U. P. Terai, intensive research has now finally incriminated *A.minimus* as the chief malaria carrier, an infectivity rate of 18 per cent having been recorded in this species at the height of the transmission season. In Orissa the field unit has collected a large amount of valuable data regarding the amount and seasonal distribution of malaria, and the bionomics of the local anopheline mosquitoes.

As regards laboratory researches, further work has been done on the behaviour of monkey malaria parasites, including observations on agglutination reactions in *P.knowlesi* infections. Several thousands of mosquito blood meals have been tested by the precipitin method. The results have had an important practical bearing on the elucidation of the malaria problems both in the Wynaad and in the U. P. Terai. Several generations of *A.stephensi* and *A.fluviatilis* have been under observation in the laboratory, and extensive observations have been made regarding the development of the ovarian follicle in mosquitoes and on the possible factor of the oesophageal diverticula.

As regard the proposals for work during the ensuing year, the present lines of teaching and investigation will be continued and further developed. In the Wynaad and U. P. attention will chiefly be directed to the development of control measures, though in the latter area further research on certain aspects of the local malaria problem is still necessary and will be continued. In Orissa, where work only commenced a few months ago, research will be directed to the incrimination of the vector species of anopheline, the study of the bionomics of these species, epidemiological studies, the study of malaria among the local population (determination of spleen rates, parasite rates, species of parasites, intensity of infections, etc.), and the formulation of a malaria control programme based on the results of the above investigations.

Should war conditions necessitate the curtailment of the laboratory research programme, the monkey malaria strains will still be maintained, so that the work may be resumed when circumstances permit.

The committee approved the proposals put forward for favourable consideration by the Scientific Advisory Board.

(ii) Malaria Transmission enquiry.

In the absence of Dr. B. M. Das Gupta the report of this enquiry was explained by Col. Chopra.

Two thousand three hundred and fifty-eight laboratory-bred clean female *A.annularis* were fed on 12 gametocyte-carriers and immediately after feeding were exposed to four different combinations of temperature and humidity in a special air-conditioning cabinet. The investigations of this species have now been completed and the data obtained are being analysed.

About 500 laboratory-bred clean female *A. culicifacies* were fed on 14 gametocyte carriers and maintained under room conditions as well as in the air-conditioning cabinet. This work is still in progress.

The chemotherapy of antimalrial drugs was studied with human and monkey malaria. The effects of a number of antimalarial drugs

in the prophylaxis of Indian strains of malaria were determined. The results have been published.

The following programme is proposed for 1940-41 :—

1. Ivestigation into the possibility of the occurrence of an endothelial schizogony in human and monkey malaria. Smears of bone marrow of cases of human malaria obtained by sternal puncture and the smears of spleen, liver and bone marrow of *rhesus* monkeys heavily parasitised with *P.knowlesi* will be studied.
2. Treatment of nervous cases with induced malaria using both human and monkey plasmodia (in collaboration with Col. Chopra).
3. Studies on the effect of antimalarial drugs on the infectivity of the patients to the mosquitoes will be continued.
4. The effect of multiple infection in mosquitoes on the human host.
5. Pathological effects of malarial plasmodia on the insect host.
6. Bionomics of *A.culicifacies*, its longevity, mating habits in captivity, etc.

The chairman pointed out that the original object of the enquiry had been swamped by the inclusion of other lines of work. A discussion followed in which Major Mulligan suggested that Bengal was not a favourable place for conducting experiments on *A.culicifacies*. Dr. Ramsay remarked that further work was needed on the bionomics of the certain malaria-carrying anophelines of Bengal, *e.g. A.phillipinensis* and *A.pallidus*.

The Committee in recommending the continuance of the enquiry were of opinion that its activities should be directed mainly towards the problem of malaria transmission.

(iii) Blackwater fever enquiry.

The report of this enquiry was presented by Dr. Krishnan.

Cortin had been produced locally by a method which is a modification of that described by Cartland and Kuizenga. The product was found to possess a high degree of potency and on biological tests, was capable of maintaining life of bilaterally adrenalectomised mice. A quantity sufficient for the treatment of 50 cases of blackwater fever was now available. At the same time, a spectrophotometric method for the assay of a potent crystalline fraction of cortin was evolved.

Efforts were made to isolate the haemolytic principle in the blood of monkeys developing haemoglobinuria. A number of extracts made from such monkeys and from human cases of blackwater fever is now ready for testing. The haemolytic principle is assumed to be unsaturated fatty acids in nature. Unfortunately, no opportunity had arisen for testing cortin on human cases of blackwater fever in the field. Encouraging results had however followed its use in two cases treated in hospital. It was not proposed to continue this enquiry owing to the difficulty of obtaining the necessary chemical reagents under present conditions.

(iv) Investigation into the maxillary index of *A.stephensi* in Calcutta.

Mr. Senior White explained that commencing from 18th February, 1939, a total of only 77 specimens had been obtained up to October. The species has become extremely rare in Calcutta, and difficulty has been

experienced in inducing the captured specimens to deposit ova. It was not possible to determine definitely the race of each specimen dissected, but measurement of the unfertilized and malformed eggs showed that M. & B. races prevailed in almost equal proportion in Calcutta. Previous records of the maxillary index therefore refer to the two races combined.

Efforts will be made to capture as many specimens as possible up to 1st April, 1940, and the results will be published in due course.

It is not proposed to continue the enquiry in 1940-41 in view of the paucity of *A.stephensi* in Calcutta.

(v) PHARMACOLOGICAL ENQUIRY AT THE HAFFKINE INSTITUTE.

In the absence of Dr. Dikshit Lt.-Col. Sokhey presented the report of this enquiry.

The work during the current year had been confined to the investigation of the action of antimalarial drugs.

1. *Plasmoquine.* The mode of destruction of plasmoquine in the body tissues was studied. The organs chiefly responsible for the destruction of plasmoquine in the body are brain and liver. Lipoid-free extracts of organs showed that their powers of destroying plasmoquine in descending order was as follows: brain, liver, spleen, testes and kidney. The agent responsible is probably of the nature of an enzyme.

By a special technique the decomposition product of plasmoquine was recovered from liver perfused with Locke's solution containing a known quantity of plasmoquine. There is reason to believe that this product is considerably less toxic to the circulatory and respiratory systems than plasmoquine.

2. *Atebrin. In vitro* experiments showed that there was no evidence of a direct lethal action of atebrin on the parasite, as a 1 in 200,000 concentration of the drug did not destroy the parasites, while *in vivo* the concentration in the blood after therapeutic doses is in the neighbourhood of 1 in 300,000. Preliminary investigations, however, indicate that the reticulo-endothelial cells play an important role in rendering the action of atebrin on malarial parasites effective.

The following proposals for the year 1940-41 were put forward:—

1. *Plasmoquine.* It is proposed to undertake a detailed pharmacological investigation of the substance produced by the destruction of plasmoquine in the body tissues with special reference to its toxicity and therapeutic activity. Attempts will also be made to determine whether the toxic action of plasmoquine, especially on the liver, could be eliminated or reduced without affecting its therapeutic value.
2. *Atebrin.* It is proposed to continue investigations into the role of the reticulo-endothelial cells in reference to the action of atebrin on parasites. The chief line of investigation will be experiments designed to block these cells in splenectomized animals. At the same time, cross circulation experiments will be conducted between monkeys infected with *P.knowlesi* and normal monkeys. This method will also be utilised to determine the site and nature of immunity, whether humoral or cellular.

Chemical investigations will also be made to determine the concentration of atebrin in normal animals and in animals whose reticulo-endothelial cells are blocked.

Col. Chopra considered that the enquiry had yielded results of considerable basic pharmacological importance.

Major Mulligan stressed the value of this work in connection with the role of the reticulo-endothelial system.

The Committee recommended the continuance of the enquiry in so far as it concerned the investigation of antimalarial drugs.

(vi) ASSAM MEDICAL RESEARCH SOCIETY (MALARIA SECTION).

Dr. Viswanathan said that a complete survey of a tea estate (Gillapukri) extending over one year had been completed.

The supervision of six malaria control projects had been continued, but no new line of research had been carried out as the post of the Research Officer was vacant during the greater part of the year. While the value of pre-monsoon and cold weather antilarval control was, on the whole, fairly well demonstrated, it was difficult to exclude the possibility that the recorded improvement in spleen rates may have been occasioned by a natural decline in the disease. A more detailed survey of Gauhati town and of Shillong is therefore considered necessary.

With regard to the control of rural malaria, the results obtained in the Surma and Brahmaputra valleys showed a satisfactory reduction in the disease. It is proposed, however, to confirm the recorded improvement by a study of adjoining villages not under such control. As the villagers failed to co-operate in abstaining from blocking the streams with embankment no further reduction in the incidence of the disease may be anticipated except at a prohibitive cost. It was therefore proposed to inaugurate an experimental scheme for the control of malaria by insecticidal spraying in villages in the Brahmaputra valley.

The Doom-Dooma cinchona-plasmoquine treatment scheme had been continued for the seventh year. The reduction in spleen and parasite rates could not be attributed solely to the treatment scheme as some antilarval work had also been carried out in this area since 1934. Another treatment scheme jointly financed by the Government of Assam and the Local Board concerned has been carried out in two groups of villages in the Surma and Brahmaputra Valleys. In this scheme cinchona reinforced with plasmoquine was given only to cases of malarial infection detected by a single thick film examination. It is proposed to revise these schemes in the ensuing year so as to provide treatment of actual fever cases throughout the period of malaria season.

In addition to the supervision of the above schemes technical guidance was afforded to 18 provincial antilarval schemes, to control schemes in 5 tea estates and to the malaria control project in Lumding under Assam Bengal Railway.

The Committee expressed its appreciation of the work reported on.

(vii) PROTOZOAL PARASITES ENQUIRY.

An account of the work of this enquiry was given by Major Mulligan.

The results obtained from researches on passive immunity in protozoal diseases indicate that the course of *P.knowlesi* and *P.cynomolgi*

infection in clean *sinicus* monkeys can be modified by the injection of plasma and spleen juice obtained from monkeys suffering from acute, sub-acute and chronic infections. The protective antibodies apparently occur in higher concentration in splenic juice than in plasma. Similar experiments on white rats infected with *Trypanosoma evansi* showed that while plasma had some protective action, spleen juice had little, if any.

Another series of experiments was undertaken to determine the duration of acquired immunity in monkey malarial infections. The amount and duration of the 'residual' immunity was found to be variable, depending chiefly upon the amount of antigenic stimulation caused by the original infection. The results are in the course of preparation for the press and it is not proposed to continue experiments of this kind.

Some preliminary experiments were carried out on the influence of well balanced as compared with deficient diets on the course of protozoal infections in collaboration with the Nutrition Research Laboratories, Coonoor. In the case of white rats, the course of infections with *T.lewisi* and *T.evansi* did not differ materially in well-fed as compared with ill-nourished animals. The above forms of protozoal infections were, however, unsuitable, as the former produced too mild and the latter too severe an infection to yield data of sufficient value. Preliminary experiments of a similar nature on simian malaria indicate that *P.cynomolgi* runs as severe a course in well-fed monkeys as it does in animals kept on a deficient diet from nine months to a year

A species of *Plasmodium* has been found to occur in the blood of the Malabar squirrel, a full description of which is now being prepared for publication. All attempts to establish this infection in common laboratory animals such as the white rat, the rabbit, the guinea-pig and the monkey have been unsuccessful, but efforts are being made to transmit it to the common tree-rat or to some other small laboratory animal.

It is proposed to follow up the preliminary results obtained in regard to passive immunity in protozoal immunity by a comparative study of the efficacy of blood plasma and spleen juice in all stages of malarial infections in monkeys and of blood plasma and lung extracts in trypanesome infection. Investigations will also be made to throw further light on the specificity of the protective bodies concerned and to isolate and to concentrate, if possible, the potent fraction. The protective action of such biological products will be linked with the action of specific drugs. The investigations on the influence of diets on the course of protozoal infections will be further extended.

The Committee recommended the continuance of the enquiry in so far as it concerns the research on malaria parasite.

PROPOSALS FOR NEW ENQUIRIES.

(i) *Enquiry on a species of avian malaria by Dr. B. C. Basu.*

A species of avian malaria (P.heroni) was found in a paddy bird, *Ardeola grayi*. The course of the disease was noticed to be of considerable severity causing a heavy mortality among birds. The disease was not of a relapsing character and recovery was followed by absolute immunity. *A.stephensi, A.subpictus, A.annularis* and *Aedes aegypti* were not susceptible to infection with this plasmodium while in the case of *Culex fatigans* the development reached oocyst stage only.

It is proposed

(a) to work out experimental transmission by mosquitoes, particularly in the different species of *Theobaldia* ;

(b) to study the exo-erythrocytic schizogony of the parasite in the capillaries of the brain ;

(c) to study other species of avian malaria ;

(d) to undertake an investigation with the chemotherapy of Indian bird malaria and

(e) to study the mechanism of relapse in avian malaria.

After considerable discussion the Committee recommended the enquiry for favourable consideration by the Scientific Advisory Board, but considered that its scope should be limited to the completion of Dr. Basu's work on *P.heroni* (items (a) and (b) of the proposals).

(ii) *Enquiry on the adult habits of A.varuna.*

Mr. Senior White explained that larval and adult collections in Singhbhum and around Chilka Lake showed that the percentage of adult *A. varuna* collected from habitations was considerably less than the proportion of its larvae found in those localities. This feature is noticeable even in a more pronounced form in the Satpuras (Central Provinces), while it is conspicuously absent in the Jeypore Hill Tracts.

As the infection rate in *A.varuna* is high amongst those captured, the extent of the damage caused by this species in these regions may be greater than is generally recognized.

The proposal is :—

(*a*) to capture all the resting mosquitoes in a number of huts in the Satpuras ;

(*b*) to make collections from a trap-net at stated hours.

By this means information would be obtained concerning the daytime resting places of the species and its true density.

The Committee were of opinion that this proposal was of considerable importance and recommended it for favourable consideration by the Scientific Advisory Board.

The resolution on malaria passed by the 16th Medical Research Conference was considered by the Committee, which noted with satisfaction that appropriate action had been taken on it.

The chairman outlined the present position regarding the control of rural malaria in India. He drew special attention to an article by Dr. Banerjea on quinine distribution in the U. P. which was appearing in the December issue of the Journal of the Malaria Institute of India. He then gave an account of the rural schemes now in progress in the various provinces. A general discussion followed and it was agreed that, apart from the provision of an adequate supply of cinchona alkaloids, the spraying of dwellings with pyrethrum insecticides, together with certain methods of naturalistic antilarval control, offered the best chances of success in combating rural malaria.

The chairman explained that an enquiry into the question of cinchona cultivation in various parts of India had been completed by Dr. Wilson and that a scheme based on his report had been considered at a recent meeting of the Advisory Board of the Imperial Council of Agricultural Research.

The Committee noted with satisfaction that this important question was being actively pursued by the Government of India.

REPORT OF THE NUTRITION ADVISORY COMMITTEE.

The Committee met on 18th December, 1939.

1. THE PROGRESS OF WORK ON THE NUTRITIVE VALUE OF FOOD-STUFFS.

The Committee took note of a report by Captain Passmore on this subject, which included a list of references to papers on food analysis.

In many Provinces there are a number of important and commonly consumed food-stuffs, not included in the existing tables, which can with advantage be analysed. As regards the future programme of research work, special attention should be given to food factors about which insufficient data are available. These include the various members of the vitamin B2 complex, and vitamins K and P. The existence of these vitamins has been demonstrated by animal experiments; their importance on human nutrition and their distribution in food-stuffs needs further study. Knowledge of the distribution of vitamins D and E in Indian foods was also incomplete.

2. PROGRESS OF DIET SURVEY WORK.

The Committee took note of the progress of diet survey work throughout India. More than 50 surveys have now been carried out in various parts of the country and the information already available about dietary habits in India is considerable. The investigations have been carried out by field workers under the additional nutrition enquiry, by officers trained in the Coonoor Laboratories and various other workers. Investigations of this nature should be continued in order to make the picture of dietary habits in India more complete. Meanwhile the data already available relating to the food-stuff composition of diets should be utilised as a guide to agricultural policy. Accordingly the note prepared by Dr. Aykroyd, expanded to include the results of further surveys, should be made available for Agricultural and Animal Husbandry Departments. The progress of diet survey work should be reviewed at the next meeting of the Committee, when the question of preparing a composite memoir or bulletin should be considered.

3. THE TRAINING OF HEALTH OFFICERS, ETC. IN COONOOR.

The Committee noted that nutrition training classes for health officers and others had been held in Coonoor in 1937, 1938 and 1939. The results have as a whole been satisfacory; the scheme has contributed to a knowledge of nutrition problems in India and to the education of the people about diet. Students attending the classes are working as full time nutrition officers in Baroda, Bengal, Bihar, Burma and the Punjab and in other Provinces and States useful work has been done by officers who are carrying out nutrition work in addition to their other duties. Provincial and State Governments should encourage public health nutrition

work as far as circumstances permit, and in this connection utilise the services of officers who have attended the training course.

4. THE COLLABORATION OF THE NUTRITION RESEARCH LABORATORIES IN THE NUTRITION WORK OF THE LEAGUE OF NATIONS.

The Committee noted that satisfactory progress in collaboration had been achieved and considered that collaboration should be continued as far as is possible in the present circumstances.

5. HEALTH BULLETIN ON RICE.

The Committee approved the health bulletin on 'Rice' prepared in the Nutrition Research Laboratories, Coonoor, and recommended that this should be published as a health bulletin at as low a price as possible.

6. RED PALM OIL.

The Committee took note of a progress report from the Harcourt Butler Technological Institute, Cawnpore, on an investigation of the possibility of using red palm as a source of pro-vitamin A for "vitaminising" edible vegetable oils or edible vegetable fats like "vanaspati". Further information about the progress of this investigation will be available at the next meeting.

7. THE WORK OF THE LIAISON OFFICER.

The Committee took note of the report of the Liaison Officer on the progress of his work. A more clearly defined programme for this officer is necessary.

8. NUTRITIONAL STUDIES IN THE PUNJAB.

The Committee expressed its approval of the reports entitled "Report on an enquiry into diets, state of nutrition and factors associated therewith in relation to health in the Kangra Valley, Punjab" and "Results of an enquiry into diets and the nutritional state of families in a famine area in Hissar District, Punjab", circulated by the Director of Public Health, Punjab. The recommendations contained in Kangra Valley report should be of great value in attacking the serious problems of malnutrition in that area. Field investigations of this nature, undertaken with the help of health officers trained in nutrition work, should be extended to other areas throughout India.

9. EXISTING AND PROPOSED ENQUIRIES.

The Committee recommended the continuance of the following existing enquiries :—

(*a*) The Nutrition Research Laboratories, Coonoor, and the Additional Nutrition Enquiry.

(*b*) Ascorbic acid enquiry by Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

(*c*) Nutritional investigation of Bengal fish by Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

(*d*) Studies on human metabolism with Indian diets by Dr. K. P. Basu, Dacca University,

(*e*) Assessment of malnutrition of Bengali boys with regard to vitamin B_1 and C by Professor N. M. Basu, Presidency College, Calcutta.

(*f*) Assessment of malnutrition of Bengali boys with regard to vitamin A deficiency by Professor N. M. Basu, Presidency College, Calcutta.

(*g*) Investigation on calcium metabolism in health and disease by Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

(*h*) Tissue culture enquiry by Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta. It is understood that this work may in due course be absorbed into the routine work of the Institute.

(*i*) Enquiry on cheap well-balanced diets under the Bombay Presidency Baby and Health Week Association. This enquiry will be continued up to the end of May 1940. A reduced staff is necessary for a further three months after this date in order to complete the report.

(*j*) Researches on the working efficiency of Indian labourers by Professor W. Burridge at the King George's Medical College, Lucknow. It is recommended that this enquiry should be continued for one month to the end of April 1940.

The continuation of the following enquiry in the coming year has not been proposed.

Investigation on basal metabolism in children and adults in the Bombay Presidency by Professor S. P. Niyogi at the Seth G. S. Medical College, Bombay.

The continuation of the following enquiries was not recommended:—

(*a*) Enquiry into the detection of adulteration of foodstuffs by the Public Analyst, Assam.

(*b*) Investigation into the problem of dental caries in India by Dr. Rahman Siddiqui, United Provinces.

The following new proposals were recommended :—

(*a*) Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal by Lt.-Col. E. O.'G. Kirwan, I.M.S., Professor of Ophthalmic Surgery, Medical College, Calcutta. It is suggested that this investigation should include a survey into the causes of blindness.

(*b*) Enquiry on the seasonal variation of the vitamin A content of the liver oil of sea-fish and the effect of parching on the biological value of proteins of cereals and pulses in the Physiology Department of Seth G. S. Medical College, Bombay.

The following new proposals were not recommended :—

(*a*) Nutritional investigations on vegetables and sweetmeats by Dr. P. Neogi, Presidency College, Calcutta.

(*b*) Investigation for ascertaining the basal metabolism of women in the United Provinces by the Professor of Physiology, King George's Medical College, Lucknow.

REPORT OF THE LEPROSY ADVISORY COMMITTEE.

The Committee met on 19th December, 1939.

I. LEPROSY ENQUIRY UNDER DR. R. G. COCHRANE.

Dr. Cochrane presented the report of his enquiry which includes clinical, dietetic, therapeutic and epidemiological studies and animal experimentation. Further data have been collected to show that the addition of skimmed milk to the diet of children accelarates the progress of lepromatous cases under treatment. Substitution of wheat diet for rice has been found to be of great value in relieving the chronic nerve pain in leprosy. Rubrophen and Fouadin have been tried in the treatment of leprosy. The value of Rubrophen, an aniline dye, in the treatment of the disease has not been confirmed. Fouadin, a pentavalent antimony compound has been found to be of value in the treatment of acute lepra reaction. The epidemiological studies consist of studying the lesions in children and of a rural investigation centre.

Dr. Cochrane made proposals for continuation of the enquiry on the same lines for the year 1940-41. The Advisory Committee recommended the continuance of the enquiry.

II. LEPROSY ENQUIRY UNDER DR. JOHN LOWE.

Dr. Dharmendra presented the report of the enquiry which includes clinical, bacteriological, immunological and biochemical studies and animal experimentation. Attempts to cultivate the organisms of leprosy and of rat leprosy have not not been successful. The work of Adler regarding the susceptibility of Syrian hamsters to human leprosy has not been confirmed.

Proposals were made for the continuation of the enquiry on similar lines for the year 1940-41. The Advisory Committee recommended the continuance of the enquiry.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met on the 19th December, 1939, and considered the reports of existing enquires and proposals for next year.

PLAGUE RESEARCHES AT THE HAFFKINE INSTITUTE, BOMBAY.

(a) Serology of plague.

(*i*) A method was developed for the preparation of an antigen suitable for agglutination tests. By its use it was shown that there was no quantitative correlation between the agglutinative titre of a serum and its protective value as determined by the mouse protection test.

(*ii*) The whole of the protective substances present in the supernatant fluid of the Haffkine vaccine was found to be precipitable with acetic acid. This precipitate could be fractionated with ammonium sulphate into fractions of different protective value. It is considered that protein only is responsible for the immunizing value of the Haffkine vaccine.

(*iii*) Vaccine filtrates and the protein fractions have been used for precipitin tests to determine the value of anti-plague sera but no strict correlaton has been found between the results of such tests and those by mouse protection experiments. Further work is required on the protecting antigenic fraction.

(*b*) *Chemotherapy.*

Tests were carried out on the value of Prontosil rubrum, Protontosil soluble and M & B 693 in the treatment of plague in mice. The Prontosil preparations were not found to have any curative action. M & B 693 did not show any action at 10 times the therapeutic dose but at 50 times the therapeutic dose a certain amount of value was shown when administered up to 48 hours after infection, but not later. The combined action of M. & B. 693 and anti-plague serum is being studied.

(*c*) *Field trials of anti-plague serum.*

These were not possible in the present year but the Madras Government is providing a plague hospital in the Cumbum Valley where future trials will be possible. Twelve horses have been under immunization.

(*d*) *Plague vaccine.*

Further studies have been made on (1) optimal temperature of incubation, (2) effect of temperature on vaccine, (3) optimal pH for growth and (4) effect of buffering the medium.

Comparisons have also been made between broth and agar vaccines at different temperatures. Approximately equal protection was given by broth vaccine at 27°C and agar vaccine at 37°C.

THE CUMBUM VALLEY ENQUIRY.

The report of this enquiry was presented by Lt.-Col. Ganapathy, I.M.S. and was considered by the Committee. The epidemiological studies have been continued and also work on prevention of plague by cyanogas fumigation and by improvements in housing. A model type of rat proof house has been introduced and the specifications of this were put before the Committee. This is the only enquiry in progress in India which is at present dealing with practical measures against plague.

BACTERIOLOGICAL STUDIES ON *P.pestis* AND *P.pseudotuberculosis* AT THE CENTRAL RESEARCH INSTITUTE, KASAULI.

The Committee considered the note presented by Major S. S. Bhatnagar on the studies of the anti-genic structure of *P.pestis* and *P.pseudotuberculosis*. The results obtained have provided information on the structure of strains of different types which will be of value in relation to the selection of strains for vaccine manufacture and for assessing the factors which are of importance in a vaccine, and for determining their presence.

RECOMMENDATIONS REGARDING THE CONTINUANCE OF ENQUIRIES.

A. The Committee consider that the enquiry at the Haffkine Institute should be continued and that the lines of work to be followed should be in relation to the results of recent antigenic studies on *P.pestis* carried out at the Institute and at Kasauli. It is recommended that the protective value of sera raised by the use of strains and *P.pestis* of different

antigenic types, including the virulent, protective avirulent, and non-protective avirulent should be tested in experimental animals and that the findings obtained should be correlated with the results of serological tests and applied to the production of therapeutic serum in horses. The facilities which will be provided at the hospital now under construction at the Cumbum Valley should be utilized for trials of the value of the sera so produced.

Tests of the protective value of vaccines prepared from strains of the different antigenic types above mentioned should be carried out in animals and the results applied to the selection of strains for vaccine manufacture and the suitability of agar grown vaccines and their methods of production studied. Further work is advisable on the use of sulphanilamide compounds, with or without antiplague serum, and should, if possible, be carried out in human cases in Cumbum Valley as well as experimentally in animals.

It is recommended that a grant of Rs. 29,348 be given for the purpose, the amounts allotted being Rs. 18,348 for staff, Rs. 6,000 for contingencies and Rs. 5,000 for field trials of serum.

It is especially recommended that Major S. S. Bhatnagar, I.M.S., who, it is understood, will be posted to the Haffkine Institute, should be associated with the enquiry to assist in carrying out protection tests in relation to antigenic structure and corresponding studies.

B. The Committee consider that the work of the Cumbum Valley enquiry should receive the support of the Association and that a grant of Rs. 1,500 should be given provided that contributions are also made by the Governments of Madras and Trvancore as in previous years. Attention is drawn to the introduction of a model type of rat proof dwelling developed at the Cumbum Valley and it is considered that the plans and specifications of this would be value to public health officers in other areas.

REPORT OF THE TUBERCULOSIS ADVISORY COMMITTEE.

The committee met on 19th December, 1939.

Enquiries under Dr. A. C. Ukil.

Dr. Ukil briefly explained the lines of investigation which were now being carried under his enquiry.

(1) *Tuberculosis in relation to industry.*

He placed before the Committee the data on tuberculosis infection in 3,008 workers hitherto tested and on the incidence of the disease among 1,214 workers who have been selected for X-Ray examination on the evidence of symptoms, physical findings and/or strong tuberculin reaction. Among the 358 workers who have been so far X-rayed, evidence of active disease has been obtained in 14.5%. The results have been analysed according to age, sex, religion, and sections of the industry. Sections where workers were exposed to greater physical strain and dust inhalation

showed a higher incidence both of infection and disease, as compared with other sections.

One thousand two hundred and seventy-two dwelling houses of the workers have been surveyed, including those who live in the surrounding villages, with regard to environmental conditions, social customs and habits, economic condition, nutrition, general mortality, tuberculosis morbidity and mortality.

The contacts of 38 sources have been examined including their relatives. All the contacts of 38 sources detected have given positive tuberculin reaction. The results have been recorded in specially prepared schedules.

Dr. Ukil proposed to continue the examination of the rest of the mill population, to conduct a survey of all the relatives and contacts of the workers, to continue the examination of contacts of 'definite' and 'probable' cases especially those living in contiguous villages, and to examine 400 new entrants to the industry. If time permitted he also proposed to enquire into the conditions prevailing in some other industries with special reference to tuberculosis.

(2) *Tuberculosis in "Home contacts" particularly amongst children.*

He placed before the meeting the data on tuberculous infection and disease among 917 children below 15 years hitherto examined, particularly in relation to some important factors such as age, source of contact, closeness of contact and duration of contact.

It has been found that the average incidence of positive reaction has been 67.9 per cent and that of demonstratable X-Ray lesions 46.7 per cent, of whom 11.6 per cent showed active lesions, 6 per cent arrested lesions and 29 per cent tracheo-bronchial adenopathy. It has also been noticed that the average incidence of demonstratable X-Ray evidence of disease varies inversely with age. The percentage of reactors has been found to be higher amongst contacts with parenchimal lesions than among those with tracheo-bronchial adenopathy. The incidence of a combination of "arrested" and "active" lesions, and of tracheo-bronchial adenopathy, as well as the different varieties of lesions taken separately, varies directly with the intensity of the tuberculin reaction. No association was noticed between morbidity rate and infection rate, nor does any relationship seem to exist with the economic status or the general health of the contacts. Tuberculosis mortality amongst child contacts in the homes investigated was found to be 50 per cent higher than general mortality in these homes in the same age group.

Dr. Ukil proposed to continue this enquiry till the estimated number of completely examined cases reached 1,000 and then to survey a number of non-tuberculous houses, in order to obtain comparable data before final deductions were made.

(3) *Typing of tubercle bacilli from pathological materials.*

Out of 218 strains isolated (of which 120 are from non-pulmonary sources, 37 from tracheo-bronchial glands and 61 from sputum, lung tissue and pleural fluid) 212 strains have been typed, all of which have been found to belong to the *human* type. In view of the reports of increasing incidence of plumonary tuberculosis from bovine sources, Dr. Ukil proposed to include the typing of 50-60 more strains from sputa, to ascertain the position in this country.

(4) *Tuberculin surveys of certain areas investigated by Dr. Ukil* 10-12 *years ago.*

Among 329 students and 217 prisoners tested up to date with Von-pirquet test, the increase over previous figures has been 6—9 per cent among students and 27 per cent among prisoners. The investigation is being continued.

An interesting discussion took place on the significance of the intensity of tuberculin reaction in relation to disease, the influence of economic status on the incidence of tuberculosis, the field of usefulness of the guinea-pig test for detecting the presence of tubercle bacilli in pathological materials and the position about bovine tuberculosis in India.

The continuance of Dr. Ukil's enquiry was recommended.

ENQUIRY INTO THE INCIDENCE OF POSITIVE MANTOUX REACTORS AND LATENT TUBERCULOSIS IN MEDICAL STUDENTS AT THE K. E. MEDICAL COLLEGE AT LAHORE, BY MAJOR G. F. TAYLOR, I.M.S.

In the absence of Major Taylor, Dr. Ukil placed before the Committee the findings on tuberculin testing and on X-Ray and physical examination of students at Lahore.

The tuberculous infection rate has been found to be 51 per cent in the first year and gradually increasing to 98 per cent in the 5th year students. The variations in the presence and intensity of tuberculin reaction are being studied by him by periodical examinations. Three students have been found to have developed pulmonary lesions during the year under review.

Major Taylor wished to continue the work over a period of 5 years by repeating the tuberculin tests, observing their variations and by examining the students from time to time by different methods.

After an interesting discussion, it was suggested to include in the investigation a number of students from the local arts colleges. The continuance of the enquiry was recommended.

NEW RESEARCHES.

Epidemological research in tuberculosis by Dr. R. G. Cochrane.

Dr. Cochrane, who is carrying out a leprosy survey at Saidapet near Madras, proposed to carry out a tuberculosis survey in the same area in collaboration with the Madanapalle Sanatorium, the King Institute of Preventive Medicine, Guindy, and the Radiological Institute, Madras. He proposed to follow up the investigation already carried out by Dr. P. V. Benjamin and his co-workers last year, with the object of finding out the causes of the spread of tuberculosis in a typical small South Indian town and of investigating methods which could be adopted for the control of the disease.

During the discussion that followed, the usefulness of this type of work was appreciated but a doubt was expressed as to whether the Provincial Government should finance such enquiries or whether the Tuberculosis Association of India or the Indian Research Fund Association should help. It was pointed out that the I. R. F. A. expected such enquiries to be financed from provincial revenues and if and where it was so done, the I. R. F. A. could help in supplementing the provincial grants. Several members desired that the Tuberculosis Association of India should help such researches. The Chairman pointed out that the resources of

the Tuberculosis Association of India, at the present moment, did not permit of making big grants, but that an offer had just been made by the Association of a token sum of Rs. 4,000/- for one year to be placed at the disposal of the I. R. F. A., for helping such enquiries as were approved by the Chairman of the Tuberculosis Association.

The general sense of the meeting was that the I. R. F. A. should help in financing this enquiry as far as possible.

(2) *Investigation into the prevalence of bovine tubarculosis in Vizagapatam by Dr. N. G. Pandalai.*

Dr. Pandalai proposed to investigate the presence of bovine tuberculosis both amongst humans and bovines in Vizagapatam by the following methods :—

(1) bacteriological and pathological examinations of the slaughter-house bovines,

(2) bacteriological examinations of milk by rapid or direct methods as advocated by Cowan, Mathews, Maitland and others and cultural and animal inoculation methods,

(3) tuberculin test on cattle, and

(4) isolation of Mycobacterium tuberculosis from various human lesions and identification of the strains by cultural and animal inoculation methods. The types of tubercular infection among people associated with slaughter-house cattle and veterinary practice will also be investigated. Mr. Ware informed the Committee that investigation under items 1-3 referred to above would be taken up by a Special Officer who would be joining the Veterinary Department in 1940. In view of this, the Committee favoured an investigation under item 4 above, but thought that the methods of identification and typing of tubercle bacilli should be done in collaboration with a central laboratory, as the "Technique" of typing was a complicated one. The Committee generally approved the enquiry subject to these conditions.

REPORT OF THE SUB-COMMITTEE APPOINTED TO CONSIDER CERTAIN ALTERATIONS IN THE REPORT OF THE TUBERCULOSIS SURVEY COMMITTEE, I. R. F. A. (1937).

The *ad interim* report of the Survey Committee, as circulated, was adopted. It was suggested that, in view of the delay involved in improving the standards of classification incorporated in the 1937 report, the Governing Body of the I. R. F. A. should be requested to publish a revised edition with appendices 4—8 substantially as they stood with a note that they were provisional and not final. The revised report may be published, as soon as possible, without further reference to the Tuberculosis Advisory Committee.

Dr. Viswanath pointed out some of the discrepancies in the results of examination of pathological materials for the presence of tubercle bacilli by animal inoculation.

It was pointed out that, in the opinion of some pathologists in India, the guinea-pig as an animal for the diagnosis of human tuberculosis was propably not as sensitive as it was reputed to be. Pathological fluids

like serous effusions and pus from clinical tuberculous lesions were stated to be frequently negative as tested by guinea-pig inoculation. To ascertain if this finding is general throughout India, the Committee suggested the collection of data from all such laboratories in India as carried out this test, particularly the various research institutions and pathology departments of teaching hospitals in India. It was proposed that the information be collected and examined statistically by the I. R. F. A. It was also suggested that the data for the last 10 years be collected and analysed. The following pro-forma was proposed for collecting the information :—

Nature of pathological material inoculated into guinea-pig.

Serial No.....

Date.....

Name.....age.....sex.....

Brief outline of clinical findings

Result of test.....

Signature.

The question of the applicability of B. C. G. vaccine in the protection of a 'Virgin' population migrating from rural to urban and industrial areas in India, was considered. General Jolly informed the committee that the Tuberculosis Association of India would probably consider the matter at the next conference of Tuberculosis Workers to be held sometime in autumn, 1940.

REPORT OF THE MATERNAL MORTALITY COMMITTEE.

The Committee met on 19th December, 1939.

1. The Committee noted that the report of Dr. Neal Edwords on the maternal mortality enquiry in Calcutta is being published as a Health Bulletin.

2. The committee considered the note of the sub-committee appointed to examine the report by Dr. J. Jhirad on maternal mortality in Bombay and heard Dr. Jhirad's remarks in reply. The committee recommended that the report in its final form should be referred to the Public Health Commissioner with a view to its publication as a Health Bulletin. The

committee recommended further that, when the reports of the three maternal mortality enquiries in Calcutta, Bombay and Delhi are published, a popular bulletin embodying the findings should be prepared.

3. (*a*) The committee noted that the memorandum on the conduct of maternal mortality enquiries had been published in the report of the Public Health Commissioner for 1937 and distributed to Directors of Public Health and other authorities and that it could be obtained on application to the Public Health Commissioner.

(*b*) The committee noted that the Director-General, Indian Medical Service, has collected information relating to maternal morbidity and mortality from institutions having more than 300 deliveries per annum. in the provinces, and that this information will be available from year to year in the future. They were informed that a summary of the returns will be included in the annual report of the Public Health Commissioner. The committee felt that in addition to this, further steps should be taken to give wider publicity to the information collected.

4. The committee heard an account by Dr. Napier of the work done in his enquiry into anaemia of women at the School of Tropical Medicine. The committee were of the opinion that the work done in this enquiry was very valuable and recommended that it should be continued.

5. Dr. Napier gave a summary of the report, which is in preparation, on the findings of the anaemia enquiry previously carried out at the Institute of Hygiene at Calcutta, and the committee noted that some useful and interesting data had been collected.

The committee were of the opinion that haemotological surveys of a similar nature were urgently needed in many parts of the country but that it was important to ensure uniformity of method and technique. It was agreed that a central laboratory, where workers could obtain special training in haemotological work, would be of great advantage.

6. Dr. Purandare gave a report of the work done in his enquiry into the toxaemias of pregnancy in Bombay and the committee recommended continuation of the work.

7. The committee heard an account by Dr. Vishwa Nath of his investigations into biochemical and dietetic factors concerned in the toxaemias of pregnancy. They recommended that the work be continued.

8. A summary of the work done in the Delhi maternal mortality enquiry was given by Dr. Neal Edwards and it was explained that, in order to complete the twelve months' work, sanction was required for an additional grant for a further two months in the current year. The additional grant was recommended.

9. Three requests for grants for new enquiries were considered :—

(*a*) *An enquiry into the bacteriology of the genital tract by Lieut.-Colonel S. N. Hayes, Lahore.* In regard to this proposal the committee felt that work on this subject was certainly needed because so little information relating to this country was available and because sepsis contributes so largely to maternal mortality. They noted with satisfaction that the services of a competent bacteriologist will be made available and that the Punjab Government were prepared to contribute a part of the cost of the enquiry.

(*b*) *A proposal for investigation into anaemia of pregnancy in the Kanara District by the Director of Public Health, Bombay.*

The committee felt that this proposal was an important one, particularly as the area was a rural one and that the work should be taken up. They recommended, however, that the research assistant selected should work under the Director, Haffkine Institute. They noted that the Government of Bombay was prepared to undertake the expenses of the enquiry apart from the pay of the research officers, for which a grant from the I. R. F. A. was requested. The committee felt that the sum estimated for laboratory appliances, medicines and contingencies etc. might fall short of the requirements.

(c) *A proposal for an investigation into pyelitis and anaemia of pregnancy by Dr. A. L. Mudaliar, Madras.*

After full consideration the committee resolved that the proposals be recommended to the Scientific Advisory Board in the order in which they are placed above.

INDIAN JOURNAL OF MEDICAL RESEARCH & INDIAN MEDICAL RESEARCH MEMOIRS.

During the year which has elapsed since the last conference Volume XXVI of the *Journal* has been completed by the publication of the January and April 1939 numbers, and the July and October numbers of Volume XXVII have appeared.

A. (1) *Volume XXVI* (1938-39).—*The Journal.*—Four quarterly numbers of this *Volume* were issued, the average size being 266 pages of literary and tabular matter and 3 plates. This represents an increase of 18 pages on the amount of matter published quarterly and a decrease in the number of plates by 3 in comparison with the previous volume. No delays occurred in publication.

(2) *The Memoirs.*—One *Memoir* was published with this volume, *viz.*, No. 30—'Studies on the Epidemiology of Filariasis in Travancore' by M. O. T. Iyengar (180 pages, 12 plates) (July 1938).

(3) *The net cost of Volume XXVI* was Rs. 14,168. This is inclusive of cost of the *Memoir* (No. 30) published with the volume (Rs. 2,849). This represents an increase in cost by about Rs. 2,000 as compared with the previous volume and is accounted for by the larger number of tabular matter pages and 4 coloured half-tone and line figures in the July 1938 issue.

B. (1) *Volume XXVII* (1939-40).—*The Journal.*—The July and October numbers have been issued containing respectively 286 pages and 11 plates and 324 pages and 13 plates. (No coloured plates have so far been published with the volume and there is little likelihood of one being published in the January or April 1940 issues).

(2) *The Memoirs.*—One *Memoir* has so far been issued with this volume, *viz.*, No. 31—'The Present Position of Hemp-Drug Addiction in India' by R. N. Chopra and G. S. Chopra (120 pages, 3 plates) (July 1939), and one (No. 32) on 'The Rice Problem in India' by W. R. Aykroyd, B. G. Krishnan, R. Passmore and A. R. Sundararajan, is in the Press (January 1940) (Approx. 100 pages).

C. *Distribution.*—

Indian subscribers	114
Foreign subscribers	161
Exchange list	173
Free list	120
Voucher copies to advertisers, file, regn:, police, make up, specimen copies to intending advertisers, propaganda, etc.	40 (approx:)
Total	608
Balance left with the publishers for sale ..	292
Total copies printed ..	900

There has thus been an increase of 17 in the distribution but the paid circulation is still very small, the increase being only 11 over last year. The exchange list has been increased by 3 by the addition of new addresses. A large number of publications is obtained in exchange for the I. R. F. A. library.

D. *Distribution lists.*—The distribution lists of the *Journal* were last revised in July 1938 and a new corrected alphabetical list showing

exchanges has been printed and copies are available from the office of the Secretary for distribution to workers under the I. R. F. A.

E. *Number of copies printed.*—This remains at 900 and it has been ascertained that there would be comparatively little saving by a reduction in the number. Spare copies of Volumes I to VIII are available from the office of the Editor, *Indian Journal of Medical Research*, Kasauli.

F. *The cost of printing the Journal.*—The following statement shows the cost of production during the last five years :—

Cost of printing the *Journal* only.

Number	XXI-XXII	XXII-XXIII	XXIII-XXIV	XXIV-XXV	XXV-XXVI
	1934-35	1935-36	1936-37	1937-38	1938-39
April	4,063	4,330	3,369	4,468	2,415
July	3,436	5,058	4,459	4,907	4 744
October	3,277	3,893	3,931	4,622	3,801
January	2,649	4,160	5,141	4,572	3652
Totals	13,425	17,441	16,900	18,369	14 612
Average pages	222	276	284	278	251
			Recoveries.		
April	1,840	1,593	1,881	1,534	2,965
July	1,105	1,593	1,381	1,534	429
October	1,017	1,659	1,381	1,185	1,048
January	1,656	371	1,466	1,185	1,048
Totals	5,618	5,216	5,983	5,438	5,490

G. *Net cost of the Journal and Memoirs.*—The following statement shows the net cost of the production of the *Journal* and *Memoirs* during the last five years :—

			Journal.		
April	2,222	2,737	1,488	2,934	--549
July	2,332	3,465	3,141	3,373	4,316
October	2,260	2,234	2,613	3,437	2,752
January	993	3,789	3,675	3,387	2,603
Totals	7,807	12,225	10,917	13,131	9,122
			Memoirs.		
				No. 29	No. 30
				2,369	2,849
Totals	..	..	..	2,369	2,849
Grand totals	7,807	12,225	10,917	15,500	11,971

For convenience of accounting all receipts are credited to the *Journal* account and the figures given for the *Memoirs* are the actual cost of production.

The net cost during 1938-39 after deducting receipts was Rs. 11,971. All Publishers' bills for 1938-39 were submitted in time to make payments within the financial year.

H. *Financial position for the year* 1939-40.—The budget allotment for the year was Rs. 13,000 for the cost of four quarterly numbers of the *Journal* plus Rs. 2,500 for any *Memoir* which might be published. A further sum of Rs. 2,500 was allotted by re-appropriation for publication of 'The Rice Problem' *Memoir* (No. 32) making a total of Rs. 18,000. Against this the actual and estimated expenditure is as follows:—

		Rs.
1.	Volume XXVI, No. 4, April 1939	1,648 (actual)
2.	Volume XXVII, No. 1, July 1939	2,584 (actual)
3.	Volume XXVII, Nos. 2 and 3, October 1939 and January 1940, @ Rs. 3,250 each	6,500 (estimated)
4.	Cost of *Memoir* No. 31	1,754 (actual)
5.	Cost of *Memoir* No. 32 .. (14,00 copies) ..	1,775 (estimated)
	Total ..	14,261 (estimated)

A saving of approximately Rs. 3,739 may be anticipated.

J. *General.*—Publication of the *Journal* has been continued on the lines laid down at the Conference of 1932 and the services of the members of the Editorial Board have been made full use of by referring to them all papers dealing with the subjects in regard to which they have been nominated.

The lines of special research activity are reflected in the nature of papers received. In volume XXVI out of 87 articles published 23 were on nutrition, 15 on biochemistry, 11 on cholera, 4 on haematology, and 3 on medical zoology. In volume XXVII so far out of 74 articles 23 were on nutrition, 12 on biochemistry, 10 on plague, 6 on cholera, 2 on haemotology, and 6 on medical zoology.

K. *Budget estimate for* 1940-41.—The following is the estimated cost of publication of the *Journal* during the year 1940-41 allowing for the usual 4 numbers and 1 *Memoir* which may possibly be received:—

4 quarterly issues of the *Journal* @ Rs. 3,250 ..	Rs. 13,000
1 *Memoir*	Rs. 2,500
Total ..	Rs. 15,500

Publications on medical research under the auspices of the Indian Research Fund Association during the year 1939.

A. *Indian Journal of Medical Research and Indian Medical Research Memoirs.*

AHUJA, M. L., and SINGH, GURKIRPAL.—Observations on the 'H' Antigen of Vibrios. (Vol. 27, No. 2).

ANDERSON, W. M. E.—Observations on *P.papatassii* in the Peshawar District. Part I. (Vol. 27, No. 2).

AYKROYD, W. R., and KRISHNAN, B. G.—A diet Survey of Families with Leprosy. (Vol. 26, No. 4).

AYKROYD, W. R., and KRISHNAN, B. G.—The Defects of Tapioca as a Staple Food. (Vol. 27, No. 1).

AYKROYD, W. R., and KRISHNAN, B. G.—A Further Experiment on the Value of Calcium Lactate for Indian Children. (Vol. 27, No. 2).

AYKROYD, W. R., KRISHNAN, B. G., PASSMORE, R., and SUNDARARAJAN, A. R.—The Rice Problem in India. (*Memoir* No. 32, January 1940).

AYKROYD, W. R., and SWAMINATHAN, M.—The Nicotinic-Acid Content of Cereals and Pellagra. (Vol. 27, No. 3).

BAGCHI, K. N., GANGULY, H. D., and SIRDAR, J. N.—Lead in Human Tissues. (Vol. 26, No. 4).

BAGCHI, K. N., GANGULY, H. D., and SIRDAR, J. N.—Lead-Content of Human Hair. (Vol. 27, No. 3).

BASU, B. C., and RAO, S. SUNDAR.—Studies on Filariasis Transmission. (Vol. 27, No. 1).

BASU, K. P., and BASAK, M. N.—Studies in Human Metabolism. Part I. Protein Metabolism in Indians. (Vol. 27, No. 1).

BASU, K. P., BASAK, M. N., and RAI SIRCAR, B. C.—Studies in Human Metabolism Part II. Calcium and Phosphorus Metabolism in Indians on Rice and on Wheat Diets. (Vol. 27, No. 2).

BASU, K. P., RAI SIRCAR, B. C., and SEN GUPTA, J. C.—Vitamin-A Content of some Species of Bengal Fish by Biological, Tintometric, and Spectroscopic Methods. (Vol. 27, No. 3).

BOSE, SARASHIPADA.—Nutritive Value of Papain-Mutton Broth. (Vol. 27, No. 1).

BOSE, SARASHIPADA.—Note on the Preparation of an Unpurified Mannose Solution for Bacteriological Use. (Vol. 27, No. 1).

CHOPRA, R. N., and CHOPRA, GURBAKHSH SINGH.—The Present Position of Hemp-Drug Addiction in India. (*Memoir* No. 31, July 1939).

CHOPRA, R. N., and RAO, S. SUNDAR.—Chemotherapy of Filarial Infection. (Vol. 27, No. 2).

CHOUDHURY, S. C., and BASU, U. P.—On Copper Content of Foods. (Vol. 26, No. 4).

DE, S. S.—Studies on Hæmolysin of Cobra Venom. Part I. Investigation on the Isolation of Hæmolysin from Cobra (*Naja Naja*) Venom. (Vol. 27, No. 2).

DE, S. S.—Studies on Hæmolysin of Cobra Venom. Part II. Effect of different Substances on the Activity of Cobra (*Naja Naja*) Hæmolysin. (Vol. 27, No. 3).

DE, S. S.—Studies on Hæmolysin of Cobra Venom. Part III. Reversible Inactivation of Hæmolysin of Cobra (*Naja Naja*) Venom. (Vol. 27, No. 3).

DHARMENDRA, and MUKHERJI, N. N.—Action of Methylene Blue on *Mycobacterium leprae muris*. (Vol. 27, No. 3).

DHURANDHAR, C. B., and BOMAN-BEHRAM, A. K.—Pigmentation of the Conjunctiva and its possible Relation to Nutrition. (Vol. 27, No. 3).

GIRI, K. V.—Indian Gooseberries (*Phyllanthus emblica* Linn.) as a Source of Vitamin C. (Vol. 27, No. 2).

GIRI, K. V., and SHOURIE, K. L.—Serum-Phosphate Activity in Rats fed on a Poor South Indian Diet variously Supplemented. (Vol. 27, No. 1).

GIRI, K. V., and SHOURIE, K. L.—The Protective Action of Tissues of Scorbutic and normal Guinea-Pigs against the Oxidation of Vitamin C and the Nature of the Protective Mechanism. (Vol. 27, No. 2).

GOYLE, A. N., and HAVILAND MINCHIN, R. L.—A Note on the Biochemical and Serological Classification of *Staphylococci*. (Vol. 27, No. 3).

KRISHNAN, B. G.—Diet Surveys in the Nilgiris and Travancore. (Vol. 26, No. 4).

LAL, R. B., and ROY, S. C.—Investigations into the Epidemiology of Epidemic Dropsy. Part VII. Further Field Studies and Controlled Experiments. (Vol. 27, No. 1).

LAL, R. B., MUKHERJI, S. P., and ROY, S. C.—Investigations into the Epidemiology of Epidemic Dropsy. Part VIII. Studies on the Nature and Origin of certain (? Toxic) Substances present in the Supplies of Mustard Oil Associated with Outbreaks of Epidemic Dropsy. (Vol. 27, No. 1).

MOITRA, N. M.—On Inhibition of Individual Types of Cholera-Bacteriophage by Vibrio Extracts. (Vol. 27, No. 1).

MAJUMDAR, B. N.—Note on the Assimilation of Carotene by Rats from a Fat-Free Diet. (Vol. 27, No. 2).

MAPLESTONE, P. A., and PANJA D.—The Complement-Fixation Test in Tuberculous Disease of the, Skin. (Vol. 27, No. 2).

MAPLESTONE, P. A., and PANJA, D.—*Molluscum contagiosum* and its Vibrios. (Vol. 27, No. 1).

MITRA, D. D.—A Study of Diet and Nutrition in North Bengal, Upper Assam, and Calcutta. (Vol. 27, No. 2).

NAPIER, L. EVERARD, SANKARAN, G., SATYA SWAROOP, and RAO, M. NARASIMHA.—Hæmatological Studies in Indians. Part X. Mean Red-Cell Diameters: Standard Curves for a Bengalee population. (Vol. 27, No. 1).

NAPIER, L. EVERARD, and DAS GUPTA, C. R.—Hæmatologial Studies in Indians. Part XI. The Choice of an Anti-Coagulant. (Vol. 27, No. 2).

NIYOGI, S. P., PATWARDHAN, V. N., and MORDECAI, J.—Studies on Basal Metabolism in Bombay. Part I. (Vol. 27, No. 1).

ORR, IAN M., and RAO, M. V. RADHAKRISHNA.—A Contribution to the Study of the Pathogenesis of Peptic Ulcer in Indians. (Vol. 27, No. 1).

RAO, M. V. RADHAKRISHNA.—Studies on Vitamin-A Deficiency. Part IV. The Effect of Carotene on the Peripheral Nerve Lesions produced by Vitamin-A Deficiency. (Vol. 27, No. 3).

READ, W. D. B.—Differential Isolation of *V.cholerae*. (Vol. 26, No. 4).

READ, W. D. B., SINGH, GURKIRPAL, SEAL, S. C., and BOSE, S.—Growth and Survival of *V.cholerae*, with Special Reference to Growth and Survival in Water. (Vol. 27, No. 1).

SAHA, K. C., and GUHA, B. C.—Nutritional Investigations on Bengal Fish. (Vol. 26, No. 4).

SEAL, S. C.—A Preliminary Note on the Relative Efficiency of Bismuth-Sulphite Medium and Peptone-Water Enrichment in the Isolation of *V.cholerae* from Human and other Sources. (Vol. 27, No. 2).

SHOURIE, K. L.—A Survey of Diet and Nutrition in Najafgarh, Delhi Province. (Vol. 26, No. 4).

SHOURIE, K. L., and SWAMINATHAN, M.—The Synthesis of Nicotinic Acid by Rats. (Vol. 27, No. 3).

SIDDONS, L. B., and ROY, D. N.—The Early Stages of *Musca inferior Stein*. (Vol. 27, No. 3).

SINGH, NARINDRA.—A Study of Diet and Nutrition in Orissa. (Vol. 27, No. 2).

SOKHEY, S. S.—Experimental Studies in Plague. Part I. Introduction. (Vol. 27, No. 2).

SOKHEY, S. S.—Experimental Studies in Plague. Part II. The Solid Medium of Choice, and the Optimal Temperature of Incubation, for the Growth of the Plague Bacillus. (Vol. 27, No. 2).

SOKHEY, S. S.—Experimental Studies in Plague. Part III. A method for determining the Number of Viable Plague Organisms in Broth Cultures. (Vol. 27, No. 2).

SOKHEY, S. S.—Experimental Studies in Plague. Part IV. Experimental Animal of Choice for Plague Work. (Vol. 27, No. 2).

SOKHEY, S. S.—Experimental Studies in Plague. Part V. A Method for Measuring the Virulence of Plague Cultures. (Vol. 27, No. 2).

SOKHEY, S. S.—Experimental Studies in Plague. Part VI. A Method for Maintaining the Virulence of *Pastereulla pestis*. (Vol. 27, No. 2).

SOKHEY, S. S., CHITRE, G. D., and GOKHALE, S. K.—The Relative Value of some Proprietory Cyanide Preparations for the Extermination of Rats and Fleas as a Plague-Preventing Measure. (Vol. 27, No. 2).

SOKHEY, S. S., and MALANDKAR, M. A.—Basal Metabolism in Indians : A Study based on the Examination of Sixty Normal Indian Men. (Vol. 27, No. 2).

STRICKLAND, C., and ROY, D. N.—Ticks found on Man. (Vol. 27, No. 1).

SWAMINATHAN, M.—The Urinary Excretion of Nicotinic Acid. (Vol. 27, No. 2).

SWAMINATHAN, M.—The Influence of Varying Levels of Calcium Intake on the Biological Value of Proteins. (Vol. 27, No. 1).

WEBSTER, W. J.—Typhus in the Simla Hills. Part IX. Laboratory Observations, chiefly on Human XK Strains. (Vol. 27, No. 3).

B. *Journal of the Malaria Institute of India.*

AFRIDI, M. K.—*Aedes* survey of Hog Island. (Vol. II, No. 1).

AFRIDI, M. K., JASWANT SINGH, and HARWANT SINGH.—Food preferences of anopheline mosquitoes in the Delhi urban area. (Vol. II, No. 3).

BASU, B. C.—Studies on the biology of the malaria parasite (*Plasmodium falciparum*). (Vol. II, No. 2).

CHOPRA, R. N., and BASU, B. C.—Studies on the effect of antimalarial drugs upon the infectivity of patients to mosquitoes. Part III. 'Prontosil'. (Vol. II, No. 2).

CHOPRA, R. N., and BASU, B. C.—Failure of 'M3' in prophylaxis of Indian strains of malaria. (Vol. II, No. 3).

COVELL, G.—Antimalaria operations in Delhi. Part I. (Vol. II, No. 1).

COVELL, G., and AFRIDI, M. K.—Antimalaria operations in Delhi. Part II. (Vol. II, No. 4).

COVELL, G., and HARBHAGWAN.—Malaria in the Wynaad, South India. (Vol. II, No. 4).

DIKSHIT, B. B.—Plasmoquine in pregnancy, with special reference to malaria. (Vol. II, No. 4).

JASWANT SINGH, and HARWANT SINGH.—Treatment of simian malaria with M. &. B. 693. (Vol. II, No. 2).

C. *List of papers published by workers under the I. R. F. A. in Journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

BANERJEE, D. N.—Culture du vibrion cholerique anarobie, les variations de son pH et son pouvoir toxique. (C. R. Soc. Biol. 1939, Vol. 130).

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BANERJEE, D. N.—Outlines of the Pathology of Cholera. (Jl. Ind. Med. Assoc. 1939, Vol VIII. 391).

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BASU, K. P., and GUPTA, K.—The role of vitamins and calcium in the utilization of proteins. (Jour. Ind. Chem. Soc. Vol. XVI. No. 9).

BASU, K. P., and GUPTA, K.—Biological value of proteins of some species of Bengal fish. (Jour. Ind. Chem. Soc. Vol. XVI. No. 10).

BASU, K. P., and HALDAR, M. K.—Supplementary relations between the proteins of pulse and those of milk. (Jour, Ind. Chem Soc. Vol. XVI., No. 4)

BASU, K. P., and HALDAR, M. K.—Biological value of the proteins of Cicer arietinum and Cajanus Indicus. (Jour. Ind. Chem. Soc. Vol. XVI., No. 4).

BASU, K. P., and MALAKAR, M. C.—Calorific value of Indian food-stuffs. (Jour. Ind. Chem. Soc. Vol. XVI., No. 9).

CHATTERJEE, H. N.—A study of the hæmolymph organs in Cholera. (J. I. M. A. Vol. VIII, May 1939, P-451).

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CHATTERJEE, H. N.—A contribution to the pathology of Cholera. (J. I. M. A., Vol. VIII, May, 1939, P-449).

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CHATTERJEE, H. N., and BASU, C. C.—Anæmia of Pregnancy in Indian Women. (The Antiseptic special diseases of women number. Vol. XXXVI, Feb., 1939, P-203).

CHATTERJEE, H. N., and BASU, C. C.—Leucocytic studies in Anæmia of Pregnancy. (J. I. M. A., Vol. IX, 1939, P-49).

CHATTERJEE, H. N., and GHOSH, S. M.—Studies in the Lecithin content of the blood of Indian women in pregnancy and under normal conditions : Presented before the 27th Session of the Indian Science Congress, Madras.

CHATTERJEE, P. K., and UKIL, A. C.—Some experiences in gold therapy in pulmonary tuberculosis. (Trans. Med. Coll. Re-Union, 10th Session Decr. 1938).

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CHOPRA, R. N., BASU B. C., and SEN, S.—Rat-bite fever in Calcutta. (*I. M. G.* Vol. 74, p. 449, 1939.)

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CHOPRA, R. N., DAS GUPTA, B. M., SEN, B., and AHMAD, Z.—Infection with Giardia Lamblia, its pathogenicity and treatment. (I. M. G., LXXIV, No. 8).

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CHOPRA, R. N., DAS GUPTA, B. M., SEN, B. and HAYTER, R. T. M.—"Prontosil" in Indian strains of malaria (I. M. G., Vol. 74, p. 321, June, 1939).

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CHOPRA, R. N., PASRICHA, C. L., GOYAL, R. K., LAL, S. and SEN, A. K.—The experimental production of syndrome of epidemic dropsy in man (I. M. G., LXXIV, No. 4).

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COCHRANE, R. G.—Recent advances in epidemiology, diagnosis and prevention of leprosy. (Journal Christian Medical Association of India, 14 (1939)).

COCHRANE, R. G., PANDIT, C. G. and MENON, K. P.—A preliminary note on inoculation of monkeys with human leprosy material after splenectomy. (International Journal of Leprosy (1939), Vol. VII, No. 3.)

COVELL, G.—Note on antimalaria operations in Delhi. (Jl. Inst. Civil Eng.).

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DAS GUPTA, B. M.—Malaria Infection in the Placenta and Transmission to the Foetus. (I. M. G., Vol. 74, p. 397, 1939.)

DAS GUPTA, B. M.—Observations on some immunological aspects of leptospiral infections. Part I. (I. M. G., Vol. 74, No. 4, p. 220, April, 1939.)

DAS GUPTA, B. M.—Observations on some immunological aspects of leptospiral infections. Part II. (I. M. G., Vol. 74, p. 617, 1939.)

DAS GUPTA, B. M.—Some anomalies in the morphology of *Plasmodium Vivax* occurring in a newborn baby. (I. M. G., Vol. 74, p. 273, No. 5, May, 1939.)

DHARMENDRA.—Re-survey of the village Debipur-hir. ("Leprosy in India" Vol. XI, No. 3.)

DHARMENDRA.—Arneth count in leprosy. ("Leprosy in India", Vol. XI, No. 4.)

DHARMENDRA and CHATTERJI, S. N.—Total excision of early neuromacular lesions. ("Leprosy in India", Vol. XI, No. 4.)

DHARMENDRA and DE, N. K.—Blood cholesterol in cases of leprosy. ("Leprosy in India", Vol. XI, No. 3.)

GHOSH, B. N., and CHOUDHURY, D. K.—Enzymes in snake venom Part V. (J. Ind. Chem. Soc., 1939, Vol. 18, p. 75.)

GHOSH, B. N., and DE, S. S.—Separation of the coagulating principle from the venom of vipera russellii. (Science and Culture, 1939, Vol. 4, p. 739.)

GHOSH, B. N., and DE, S. S.—Proteins of the Rattle snake venom. (Nature, 1939. Vol. 143, p.380.)

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LOWE, J.—Leprosy and tuberculosis. (Ind. Med. Gaz. Vol. LXXIV, Aug., 1939)

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LOWE, J. and CHATTERJI, S. N.—Scarification, tattooing, etc. in relation to leprous lesions of the skin. ("Leprosy in India", Vol. XI, No. 1.)

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MAPLESTONE, P. A. and DEY, N. C.—The use of dyes in various fungal infections. (I. M. G., Vol. LXXIV, No. 7, p. 391.)

MAPLESTONE, P. A. and SUNDAR RAO, S.—Dracontiasis or Guinea-worm Disease. (Health Bulletin No. 7, Govt. of India Press, New Delhi, 1939.)

MAPLESTONE, P. A. and SUNDAR RAO, S.—Tail of Male Wuchereria bancrofti (Records of the Indian Museum Vol. XLI, part I, p.35).

MAPLESTONE, P. A. and SUNDAR RAO, S.—Case of Gnathostomiasis with some interesting features. (I. M. G., Vol. LXXIV, No. 8.)

NAPIER, L. E.—The ætiology of tropical macrocytic anæmia. (I. M. G., Vol. LXXIV, No. 1, Jany. 1939, p 1.)

RANGANATHAN, S., and SUNDARARAJAN, A. R.—Foods and Nutrition. (Annual Review of Biochem. and Allied Research in India. 1939, Vol, IX, pp. 40-53.)

RAY, P. N. and SUNDAR RAO, S.—Chyluria of Filarial Origin. (Br. Jl. Urology, Vol. XI, p. 48.)

ROY, D. N., and SIDDONS, L. B.—On the Life history and bionomics of *Chrysomyia rufifacies*. (Macq. Parasitology, Cambridge.)

ROY, D. N., and SIDDONS, L. B.—A list of Hymenoptera of the Superfamily Chalcidoidea, parasite of *Calyptrate muscoidea*. (Records of Indian Museum, Calcutta.)

SANKARAN, G.—Tissue Culture. (Science and Culture, Vol. V, No. 5, November, 1939.)

SEN, P. K.—Pneumonoconiosis with special reference to Silicosis, Anthracosis and Tuberculosis. (I. M. G., Vol. LXXIV, No. 9)

SEN, P. K.—Relapse in cases of pulmonary tuberculosis. (Trans. Med. Coll. Reunion, 10th Session, December, 1938.)

UKIL, A. C.—Le probleme de la tuberculose chez les travailleurs de l' industrie dans l' Inde. (Bull. Office Inter. d'Hygiene publique, Tome XXXI, No. 7, July, 1939.)

UKIL, A. C.—Tuberculosis among women in Bengal. (Jour. Beng. Tub. Assocn., Vol. IV, No. 10, Aug. 1939.)

UKIL, A. C. and DE, K. N.—Bronchiectasis—its ætiology, pathology, diagnosis, prognosis and treatment. (Ind. Med. Gazette. Vol. LXXIV, No. 9, Sept. 1939.)

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on the 20th December, 1939.

	Enquiries	Budget allotments Rs.
1.	Cholera field enquiry in Bengal under Dr. S. R. Pandit ..	26,969
2.	Cholera field enquiry under the Director, King Institute, Guindy, Madras	8,795
3.	Cholera vibrios enquiry under the Director, Central Research Institute, Kasauli	300
4.	Cholera bacteriological enquiry under Major C. L. Pasricha, I.M.S. at the School of Tropical Medicine, Calcutta ..	6,800
5.	Cholera toxin enquiry under Dr. H. Ghosh at the Indian Institute for Medical Research, Calcutta	616
6.	Enquiry on cholera kidney under Dr. D. N. Bannerjee at the Carmichael Medical College, Calcutta	2,500
7.	Malaria Institute of India (Public Health Section) ..	60,500
8.	Malaria Institute of India (Research Section)	35,314
9.	Malaria transmission enquiry under Dr. B. M. Das Gupta at the School of Tropical Medicine, Calcutta	6,050
10.	Nutrition research, I. R. F. A., Coonoor, under Dr. W. R. Aykroyd	85,740
11.	Enquiry on cheap well balanced diets under the Bombay Presidency Baby and Health Week Association ..	2,041
12.	Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science and Technology, Calcutta ..	5,160
13.	Nutritional investigation on Bengal fish under Dr. B. C. Guha at the University College of Science and Technology, Calcutta	3,040
14.	Enquiry entitled 'studies on human nutrition with Indian diets' under Dr. K. P. Basu at the Dacca University ..	4,812
15.	Enquiry on the working efficiency of Indian labourers under Professor W. Burridge at the King George Medical College, Lucknow	170
16.	Enquiry into malnutrition of Bengali boys with reference to vitamin A deficiency by Professor N. M. Basu at the Presidency College, Calcutta	3,106
17.	Enquiry into malnutrition of Bengali boys with reference to vitamins B and C deficiency under Professor N. M. Basu at the Presidency College, Calcutta	2,400
18.	Enquiry on calcium metabolism in health and disease under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay	2,900

	Enquiries	Budget allotment Rs.
19.	Tissue culture enquiry under Dr. G. Sankaran at the All-India Institute of Hygiene and Public Health, Calcutta ..	2,858
20.	Additional nutrition enquiry at the Nutrition Research Laboratories, Coonoor. (*The cost of this enquiry is to be met from the Rs.* 30,000/- *special annual grant sanctioned by the Governing Body over a period of five years, out of the special ten lakhs grant made by Government for antimalaria work*)	13,425
21.	Leprosy enquiry under Dr. John Lowe at the School of Tropical Medicine, Calcutta	5,432
22.	Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leper Settlement, Chingleput, Madras ..	2,460
23.	Plague researches under the Director, Haffkine Institute, Bombay	29,348
24.	Tuberculosis enquiry under Dr. A. C. Ukil at the All-India Institute of Hygiene and Public Health, Calcutta ..	12,739
25.	Enquiry on the Mantoux test under Major G. F. Taylor, I.M.S. at the King Edward Medical College, Lahore ..	500
26.	Indigenous drugs enquiry under Bt.-Col. R. N. Chopra, C.I.E., I.M.S.(R.) at the School of Tropical Medicine, Calcutta	24,071
27.	Indigenous drugs enquiry under Dr. Khem Singh Grewal at the King Edward Medical College, Lahore	2,165
28.	Indigenous drugs enquiry under Professor A. S. Paranjpe at the Seth G. S. Medical College, Bombay	2,320
29.	Enquiry into anæmia of women in Assam under Dr. L. E. Napier at the School of Tropical Medicine, Calcutta ..	5,416
30.	Enquiry into toxæmia of pregnancy under Dr. N. A. Purandare at the Seth G. S. Medical College, Bombay ..	2,971
31.	Enquiry into biochemical and dietetic factors concerned in the toxæmia of pregnancy under Dr. Vishwa Nath at the Kind Edward Medical College, Lahore	1,200
32.	Helminthological enquiry under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta	3,522
33.	Medical mycology enquiry under Dr. P. A. Maplestone at the School of Tropical Medicine, Calcutta	7,255
34.	Filariasis enquiry under Dr. S. Sundar Rao at the School of Tropical Medicine, Calcutta	4,608
35.	Protozoal parasites enquiry under the Director, Pasteur Institute, Coonoor	9,836
36.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	500
37.	Epidemic dropsy enquiry (field section) under Dr. R. B. Lal at the All-India Institute of Hygiene and Public Health, Calcutta	2,868

	Enquiries	Budget allotment Rs.
38.	Kala-azar enquiry under Dr. R. O. A. Smith at the Medical School, Darbhanga	7,620
39.	Pharmacological enquiry under Dr. B. B. Dikshit at the Haffkine Institute, Bombay	5,968
40.	Enquiry on the algae present in water supply reservoirs and their effect on purification methods and on public health under the Director, King Institute, Madras	2,692
41.	Enquiry into endemic fluorosis under the Director, King Institute, Guindy, Madras	3,888
42.	Enquiry into isolation of the different constituents of snake venoms with a view to determining their chemical nature under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta	2,800
43.	Enquiry into the ætiology of infantile biliary cirrhosis with special reference to its anæmia under Dr. Ramachandra Rao at the Medical College, Madras	2,520
44.	Enquiry into filariasis under Dr. T. Bhaskara Menon, Professor of Pathology, Medical College, Vizagapatam ..	1,860
45.	Peptic ulcer enquiry under Captain J. R. Dogra, I.M.S., at the King Institute, Guindy, Madras	1,621
46.	Enquiry on a species of avian malaria under Dr. B. C. Basu at the Imperial Veterinary Research Institute, Mukteswar	1,000
47.	Enquiry on the adult habits of *A.varuna* under Mr. R. Senior White, Malariologist, B. N. Railway, Calcutta ..	544
48.	Enquiry on nutrition and its bearing on preventable blindness and eye diseases in Bengal under Lt.-Col. E. O. G. Kirwan, Professor of Ophthalmic Surgery, Medical College, Calcutta	2,000
49.	Epidemiological enquiry on tuberculosis under Dr. R. G. Cochrane, Chief Medical Officer, Lady Willingdon Leper Settlement, Chingleput, Madras	4,000
50.	Enquiry into the prevalence of bovine tuberculosis in Vizagapatam under Dr. N. G. Pandalai, Professor of Bacteriology, Medical College, Vizagapatam	2,040
51.	Enquiry on the bacteriology of the genital tract under Lt.-Col. S. N. Hayes, I.M.S., Medical Superintendent, Lady Willingdon Hospital, Lahore	2,170
52.	Investigation into the anæmia of pregnancy in Kanara District under the Director of Public Health, Bombay ..	3,240
53.	Enquiry on various virus diseases with particular reference to variola vaccinia under Dr. R. K. Goyal, School of Tropical Medicine, Calcutta	1,400
54.	Enquiry on tetanus under Dr. B. B. Yodh, Lamington Road, Bombay	2,000
55.	Enquiry on the seasonal variation of the vitamin A content of liver oil of sea-fish and the effect of parching on the	

	Enquiries	Budget allotment Rs.
	biological value of proteins of cereals and pulses in the Physiology Department of Seth G. S. Medical College, Bombay	1,750
	Grants	
(*i*)	Administration charges	30,809
(*ii*)	Contributions towards the cost of two professorships at the School of Tropical Medicine, Calcutta	17,505
(*iii*)	Contribution towards the cost of the plague enquiry under the Madras Government in the Cumbum Valley ..	1,500
(*iv*)	Contribution to the Imperial Institute of Entomology, London	1,350
(*v*)	Contribution to the Bureau of Hygiene and Tropical Diseases, London	2,025
(*vi*)	Contribution towards the cost of the Statistical Bureau under the Public Health Commissioner with the Government of India	5,700
(*vii*)	Indian Journal of Medical Research and Memoirs	
	(*a*) Publication charges of the Journal Rs. 13,000	
	(*b*) Publication charges of the Memoirs Rs. 2,500	
	(*c*) Editor's office .. .. Rs. 4,056	
		19,556
(*viii*)	I. R. F. A. Library at the Central Research Institute, Kasauli	2,140
(*ix*)	Pay and contributions of the officers of the Medical Research Department under the Association	1,50,000
(*x*)	House rent, compensatory and travelling allowances and certain contingent expenses payable to the eight officers of the Medical Research Department	3,000
(*xi*)	Pay and travelling allowance on transfer of the three workers in the Junior Research cadre	17,340
(*xii*)	Pay of two clerks at the School of Tropical Medicine, Calcutta	2,820
(*xiii*)	Clerical assistance to the Director, All-India Institute of Hygiene and Public Health, Calcutta	1,077
(*xiv*)	Travelling allowance of subordinate workers on transfer from one post to another	1,000
(*xv*)	I. R. F. A. contribution and interest towards the Contributory Provident Fund	12,000

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